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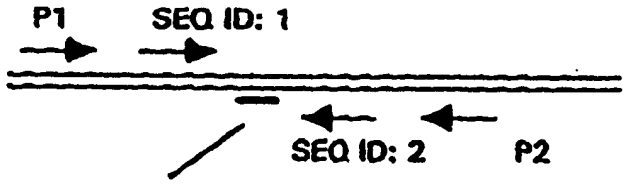

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(57) Abstract

Nucleic acid probes and primers are described for detecting fungi that cause disease in humans and animals, as well as spoilage of food and beverages. These probes can detect rRNA, rDNA or polymerase chain reaction products from a majority of fungi in clinical, environmental or food samples. Nucleic acid hybridization assay probes specific for *Acremonium* sp., *Aspergillus clavatus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus glaucus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Aspergillus unguis*, *Aspergillus ustus*, *Beauveria* sp., *Bipolaris* sp., *Blastoschizomyces* sp., *Blastomyces dermatitidis*, *Candida albicans*, *Candida glabrata*, *Candida guilliermondii*, *Candida kefyr*, *Candida krusei*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida tropicalis*, *Chrysosporium* sp., *Cladosporium* sp., *Coccidioides immitis*, *Cryptococcus neoformans var gattii* serotype B, *Cryptococcus neoformans* serotype A, *Cryptococcus laurentii*, *Cryptococcus terreus*, *Curvularia* sp., *Fusarium* sp., *Filobasidium capsuligenum*, *Filobasidiella* (Cryptococcus) *neoformans var bacillispora* serotype C, *Filobasidiella* (Cryptococcus) *neoformans var neoformans* serotype D, *Filobasidium uniguttulatum*, *Geotrichum* sp., *Histoplasma capsulatum*, *Malbranchea* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium species*, *Pseudallescheria boydii*, *Rhizopus* sp., *Sporothrix schenkii*, *Scopulariopsis brevicaulis*, *Scopulariopsis brumpti*, *Saccharomyces cerevisiae*, and *Trichosporon beigelii* are also described.

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NUCLEIC ACID PROBES FOR THE DETECTION AND IDENTIFICATION OF FUNGI

FIELD OF INVENTION

The inventions described and claimed herein relate to the design and composition of two nucleic acid probes capable of detecting many different fungal organisms in clinical, food, environmental and other samples. The inventions described and claimed herein also relate to the design and composition of probes capable of specifically detecting and identifying *Acremonium* sp., *Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Aspergillus unguis, Aspergillus ustus, Beauveria* sp., *Bipolaris* sp., *Blastoschizomyces* sp., *Blastomyces dermatitidis, Candida albicans, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Chrysosporium* sp., *Cladosporium* sp., *Coccidioides immitis, Cryptococcus neoformans var gattii* serotype B, *Cryptococcus neoformans* serotype A, *Cryptococcus laurentii, Cryptococcus terreus, Curvularia* sp., *Fusarium* sp., *Filobasidium capsuligenum, Filobasidiella* (Cryptococcus) *neoformans var bacillispora* serotype C, *Filobasidiella* (Cryptococcus) *neoformans var neoformans* serotype D, *Filobasidium uniguttulatum, Geotrichum* sp., *Histoplasma capsulatum, Malbranchea* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium species, Pseudallescheria boydii, Rhizopus* sp., *Sporothrix schenkii, Scopulariopsis*

brevicaulis sp., *Scopulariopsis brumpti, Saccharomyces cerevisiae,* and *Trichosporon beigelii* in clinical, food, environmental and other samples.

Fungi are eukaryotic microorganisms that are universally distributed. While in nature fungi play a major role in the decomposition of plant materials, they are also responsible for spoilage of food, beverage and pharmaceutical preparations. Out of an estimated 100,000 species of fungi described by mycologists, approximately 150 species are pathogenic to man and animals. The increasing incidence of AIDS and the development of newer treatments for hematologic malignancies and organ transplants has lead to an increase in the number of immunocompromised patients. These patients have a high risk of developing fungal infections, which if not rapidly diagnosed and treated are capable of causing death in a matter of days. The number of antifungal drugs is limited and their toxic side effects on the patient are much higher than that of comparable antibacterial therapy. A rapid diagnosis of fungal infection and start of treatment is critical in these patients. Books by Kwon-Chung and Bennett, along with Sarosi and Davies, provide an overview into the medical importance of fungi.

Fungal organisms are identified by morphology and nutritional characteristics. Fungi may take anywhere from two days to several weeks to grow in culture and often the same organism can take radically different forms depending on the growth conditions. This makes timely identification difficult even for the classically trained expert and impedes the treatment of patients where rapid identification of genus and species is of medical advantage.

The incidence and distribution of major pathogenic fungi varies by geographic location. *Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Coccidiodes immitis, Cryptococcus neoformans, Histoplasma capsulatum,*

Paracoccidioides brasiliensis, Pseudallescheria boydii and Sporothrix schenkii represent some of the leading causes of mycotic infections.

Aspergillus fumigatus is among the top three causes of systemic fungal infection treated in hospitals. It usually affects patients with organ transplants, acute leukemias and burns and can be rapidly fatal if not diagnosed quickly. With over 150 species of *Aspergillus* present in the soil, air and water, accurate detection of *Aspergillus fumigatus* becomes extremely important. *Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Aspergillus unguis and Aspergillus ustus* represent a majority of *Aspergillus* species seen in clinical specimens and their presence can cause diagnostic difficulties. *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger* have been linked with disease in humans, with *Aspergillus fumigatus* being the predominant pathogen in North America. A few immunologic tests exist for *Aspergillus fumigatus* but these have limited sensitivity and specificity. There are also reports of development of polymerase chain reaction based tests for *Aspergillus fumigatus* based on the amplification of the *Asp f1* antigen gene and a ribosomal intergenic spacer (Spreadbury et. al.). The Spreadbury technique is based on the PCR amplification of a 401 bp fragment spanning the large subunit rRNA/intergenic spacer region. This relies on a pair of primers to specifically amplify DNA from *Aspergillus fumigatus* only, and is of no utility in identifying other fungi.

Blastomyces dermatitidis is present in the soil, usually in bird droppings and animal feces. Infections often occur at construction sites and the ensuing lung infiltration and pneumonitis are usually fatal in immunocompromised patients. Diagnosis by culture may take weeks, and the organism is occasionally mistaken for other fungi. Existing immunological diagnostic tests are unreliable, and there is a need for rapid and reliable DNA based diagnostic tests. Similarly, *Histoplasma*

capsulatum exists in the soil and is known to have infected at least 20% of the population of North America. Most infections start in the lung and resolve spontaneously, but may occasionally spread to other organs. AIDS patients represent a growing number of cases of Histoplasmosis. Diagnosis is difficult as immunological tests are often negative during the first 4-6 weeks of infection. *Coccidioides immitis* is found in abundance in the soil in Southwestern United States. Dust storms, farming, building construction, earthquakes and even hiking have been linked with outbreaks of disease. Lung infection followed by cavitation and disseminated miliary coccidioidomycosis are seen. Meningitis is usually lethal, and as with other fungi, mortality is highest in debilitated hosts. Four serotypes of *Cryptococcus neoformans* cause disease in humans. These are *Cryptococcus neoformans* serotype A, *Cryptococcus neoformans* var *gatti* serotype B, *Filobasidiella (Cryptococcus) neoformans* var *bacillispora* serotype C and *Filobasidiella (Cryptococcus) neoformans* var. *neoformans* serotype D. The incidence of this disease is growing rapidly, with up to 10% of HIV infected people developing cryptococcosis. DNA probes capable of detecting all 4 serotypes are required for the early diagnosis and treatment for life threatening infections like cryptococcal meningitis. A report by Stockman et. al. discusses commercial tests for *Histoplasma*, *Blastomyces*, *Coccidioides*, and *Cryptococcus* based on the 18S rRNA (Gen-Probe, Inc., San Diego, CA). The authors report sensitivities ranging from 87.8 to 100% and a specificity of 100%. One drawback of these probes is that these are used on rRNA extracted from fungal cultures. As some fungi may require up to 3 weeks to grow in culture, this technique cannot be used to expedite diagnosis until a culture becomes available.

Candida albicans is one of the most common causes of fungal infection in humans. It is present in the respiratory, gastrointestinal and female genital tract of healthy individuals, and acts as an opportunistic pathogen in debilitated individuals on steroid or chemotherapy. Diabetes mellitus and indwelling catheters are other

predisposing causes. Immunocompromised hosts show rapid hematogenous spread of fungi. Morbidity and mortality in untreated cases is high. *Candida glabrata*, *Candida guilliermondii*, *Candida kefyr*, *Candida krusei*, *Candida lusitaniae*, *Candida parapsilosis* and *Candida tropicalis* are also known to cause disease in humans. DNA probes capable of identifying these individual species would eliminate the need for multiple blood cultures and lengthy biochemical speciation.

Recent advances in molecular techniques have led to the approach of microbe detection and identification based upon the DNA sequence of ribosomal genes. Commonly used detection techniques include either direct amplification of the ribosomal DNA (rDNA) genes by the polymerase chain reaction, or reverse transcription of the ribosomal RNA (rRNA) into complementary DNA (cDNA) followed by polymerase chain reaction amplification of the cDNA. Ribosomes are composites of unique rRNA and protein species that function in the translation of messenger RNA into protein. Evolutionary studies are consistent with the interpretation that all extant life has evolved from a single organism. Thus, all cellular organisms contain rRNA and these rRNAs are related by evolution. The evolutionary process is such that each species of organism appears to have unique regions of sequence in its ribosomal genes. The presence of these unique species specific regions allows one to design DNA probes that under conditions of hybridization will specifically bind to, and identify the polymerase chain reaction amplified DNA from only one species of fungus. For the purposes of this application, the word "primer" is used to mean a nucleotide sequence which can be extended by template-directed polymerization, and "probe" is used to mean a nucleotide sequence capable of detecting its complementary sequence by hybridization. Also, for the purpose of this application, the phrase "nucleotide sequence" is intended to include either DNA or RNA forms or modification thereof. Furthermore, those versed in the art will recognize that primer sequences can be used as probes and vice versa. The use of nucleic acid hybridization to detect

specific nucleic acid sequences of interest is also described by Kohne (U.S. Patent 4,851,330, 7/1989).

In prokaryotes and eukaryotes, ribosomal RNA and the corresponding rDNA genes are identified by the size of the RNA. The sizes are related in terms of sedimentation velocity or S values. Thus, for prokaryotes the values are 5S, 16S, and 23S; and for eukaryotes the values are 5S, 5.8S, 18S and 28S. Because all ribosomes perform the same function which is essential for cell viability, ribosomal sequences are largely conserved, yet certain regions of each ribosomal species are subject to more variation without consequence to function. It is these hypervariable regions that allow one to identify different species amongst members of the same genus. As noted in the references, there are several reports where 5S, 18S and the intergenic spacer between 5.8S and 28S rDNA have been used for the detection and identification of fungi (Holmes et. al., Hopfer et. al., Lott et. al., Maiwald et. al., Makimura et. al., Mitchell et. al., Nakamura et. al.). Holmes et. al. describe a PCR test based on the co-amplification of the 5S rDNA and an adjacent nontranscribed spacer region. This identifies only *Candida albicans* and detects other *Candida* species without identifying individual organisms. Hopfer et. al. and Maiwald et. al. both use universal primers to amplify 18S rDNA from several fungi including *Candida* sp., *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Trichosporon* sp. These amplicons are digested with restriction enzymes and the cut fragments are sized by gel electrophoresis. This restriction fragment length polymorphism pattern enables them to identify most but not all organisms. This technique can be used on amplified DNA from a pure fungal culture. As clinical samples such as sputum usually contain multiple fungal organisms, this technique has little utility in diagnosis as multiple overlapping fragments obtained from a mix of fungi would be nearly impossible to interpret. Lott et. al. use the 5.8S RNA and the internal transcribed spacer (ITS2) to identify and speciate *Candida albicans* and related *Candida* species. Makimura amplifies a 687 bp fragment from the 18S rDNA of 25

medically important fungi and uses these in the diagnosis of *Candida albicans* in clinical samples. Mitchell uses nested PCR to amplify 5.8S and internal transcribed spacer (ITS) to identify *Cryptococcus neoformans*. No subsequent testing is done to verify the identity of the amplified DNA. Nakamura et. al. use 18S primers to detect *Aspergillus fumigatus* infections of the lung. Most protocols given in these references can only be used to detect an extremely limited number of fungi from a clinical specimen. Hopfer et. al. and Maiwald et. al. can identify multiple organisms from pure cultures, but their utility for clinical specimens containing multiple fungal species is limited at best.

United States patents have been issued to Weisburg et. al. for probes developed for the detection of 18S small subunit ribosomal RNA sequences in fungi. These probes will detect fungi from many species, but cannot be used easily to identify any single species. United States patents have also been issued to Milliman for probes developed for the specific detection of the bacteria *Staphylococcus aureus* based on the 16S ribosomal sequences. Hogan et. al. (European Pat. App. 0,272,009) describe one fungal probe for 18S rRNA and three fungal probes for 28S rRNA sequences. Two of these 28S probes detect several different fungi while the third probe detects *Candida krusei* from a limited panel of 10 fungi. None of the 28S probes described by Hogan et. al. is related to any of the probes described in our invention. All probes claimed in our invention can be mapped within the first 900 base pairs of a 28S gene. The probes described by Hogan et. al. are located further 3' on the 28S sequence, between base pairs 1000 and 2000 (these numbers are comparable to the primary sequence of *Saccharomyces cerevisiae* 28S rRNA gene. Genbank accession number: J01355). Leclerc et. al. have published reports analyzing the phylogenetic relationship between fungi based on partial DNA sequences of several fungal 28S genes sequenced by them. Some of the organisms claimed to have been sequenced by Leclerc are the same as some organisms sequenced by us. These are *Sporothrix schenckii, Pseudallescheria*

boydii, Blastomyces dermatitidis, Histoplasma capsulatum and Chrysosporium sp. Leclerc et. al. have not published any sequence data in their report, and to the best of our knowledge, they have not made these sequences publically available in the GenBank. The reverse-complement sequence of their sequencing primer 401 (TCCCTTTCAA CAATTTCACG) overlaps our SEQ ID NO: 1 (GTGAAATTGT TGAAAGGGAA) by 19 nucleotides and their sequencing primer 636 (GGTCCGTGTT TCAAGACGG) overlaps our SEQ ID NO: 2 (GACTCCTTGG TCCGTGTT) by 10 nucleotides. We are aware of no reports in the literature of variable regions from 28S rRNA genes of fungi being used as targets for the development of species specific diagnostic probes.

As discussed above, most present techniques for the molecular detection of fungi rely on the use of highly specific primers for the PCR amplification of only one fungal species. Those that employ "Universal" primers for a PCR amplification of DNA from multiple organisms, use post-PCR amplicon identification techniques that are useful only on pure cultures of fungi. These are not be able to identify fungi from a clinical specimen containing multiple fungal organisms. Our first aim was to develop "Universal" primers for the 28S gene. These primers would be capable of amplifying in a PCR, 28S rDNA from most fungi. Our subsequent aim was to develop species specific probes for fungi of interest, that would be used to analyze our "Universal" 28S amplicon. These species specific probes would be able to detect the presence of fungi of interest even in situations containing mixed fungal species.

One aspect of this invention is to provide nucleic acid primers capable of detecting 28S sequences from DNA or RNA of most fungi. These would be used as "Universal" primers in a polymerase chain reaction to amplify 28S sequences from any fungus present in clinical, food, environmental or other samples. These "Universal" primers would also be used to sequence the amplified DNA. The

sequence obtained would be used to identify the fungus by comparing with a database of known fungal sequences.

A second aspect of this invention is to provide nucleic acid probes capable of detecting and identifying, by nucleic acid hybridization, the pathogens *Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, Aspergillus flavus, Aspergillus glaucus, Aspergillus niger, Aspergillus terreus, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Pseudallescheria boydii, Sporothrix schenckii* and other species by use of any of several different formats. Additionally, nucleotide sequence information is provided to identify these pathogens and other fungi by DNA sequence comparison (Figure 2) or by the construction of additional probes.

SUMMARY OF THE INVENTION

Nucleic acid probes and primers are described for detecting fungi that cause disease in humans and animals, as well as spoilage of food and beverages. These probes can detect rRNA, rDNA or polymerase chain reaction products from a majority of fungi in clinical, environmental or food samples. Nucleic acid hybridization assay probes specific for *Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, Aspergillus flavus, Aspergillus glaucus, Aspergillus niger, Aspergillus terreus, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Pseudallescheria boydii, Sporothrix schenckii* and other species (Table 1 and Figure 2) are also described.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents the relative position of the sequences described on the 28S subunit of fungi.

Figures 2A, B, C and D together represent the multiple sequence alignment for (SEQ ID NO: 24) through (SEQ ID NO: 74).

DETAILS OF THE INVENTION

Our first objective was to develop nucleic acid primers for use in a polymerase chain reaction to amplify 28S genes from all fungi likely to be present in a clinical sample. This amplified DNA would then be amenable to probing with several different species specific probes. Each one of these species specific probes would, under conditions of hybridization, anneal to 28S ribosomal DNA from only one species of fungus, thereby detecting and identifying the species of fungus present in the clinical sample. The 28S gene was selected as a target because it had regions that were conserved among fungi and these would provide potential annealing sites for "universal" fungal probes. The ribosomal 28S genes were also expected to have hypervariable regions that would be unique enough to provide sites for species specific probes. The large rRNA gene is called the 23S rRNA gene in prokaryotes and 28S in eukaryotes. This designation is based on the length and therefore the sedimentation coefficient of these rRNA molecules. Fungal large subunit rRNAs vary in size among different organisms and are often referred to as being 25S, 26S or 28S. Since fungi are eukaryotes, and to maintain uniformity in this application, we shall refer to fungal large subunit rRNA as 28S rRNA.

Published sequences from *Cryptococcus neoformans*, two *Candida albicans*, *Saccharomyces cerevisiae* and two *Schizosaccharomyces pombe* 28S genes are approximately 3.5 kilobases in length (Genbank accession numbers:

L14068, L28817, X70659, J01355, Z19136 & Z19578). These four sequences were aligned, and a region of sequence variability was found clustered between coordinates 200 and 700 from the 5' end of these genes. As an initial starting point, two nucleic acid primers P1 (ATCAATAAGC GGAGGAAAAG) and P2 (CTCTGGCTTC ACCCTATTC) (see figure 1), capable of hybridizing to all 4 of the above mentioned organisms and not to human 28S sequences (GenBank accession number: M11167), were designed and used under low stringency hybridization conditions in a polymerase chain reaction to amplify approximately 800 base pairs of DNA spanning this hypervariable region from the following 34 fungi that were obtained from the Mayo Clinic fungal collection: *Acremonium* sp., *Aspergillus clavatus, Aspergillus fumigatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Aspergillus unguis, Aspergillus ustus, Beauvaria* sp., *Bipolaris* sp., *Blastomyces dermatitidis, Candida albicans, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Chrysosporium* sp., *Cladosporium* sp., *Coccidioides immitis, Cryptococcus neoformans* serotype A, *Curvularia* sp., *Geotrichum* sp., *Histoplasma capsulatum, Mucor* sp., *Penicillium* sp., *Pseudallescheria boydii, Saccharomyces cerevisiae, Sporothrix schenkii and Trichosporon beigelii.*

DNA was extracted from the fungi listed above by the following method. A loopful of fungal culture was scraped off a culture plate using a sterile inoculation loop. The fungus was added one milliliter of sterile water in a 1.5 ml Sarsted (Newton, North Carolina) screw cap microcentrifuge tube. This tube was placed in a boiling water bath for 20 minutes in order to lyse the fungus and release DNA from the cells. Two microliters of this whole cell lysate was used in a PCR to amplify 28S rDNA. All PCR amplifications were carried out as hot-start reactions in a 50 ul reaction volume using Perkin-Elmer (Norwalk, CT) 0.5 ml thin-wall polypropylene tubes and a Perkin-Elmer thermal cycler. Reagents added to the tube

initially were 2.5 ul of 10X PCR buffer (100 mM tris pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 5.0 ul of 50% glycerol/1 mM cresol red, 8.0 ul of dNTP mix (1.25 mM each of dATP, dGTP, dTTP and dCTP), 12 picomoles of each nucleic acid primer and sterile water to make up a volume of 25 ul. A wax bead (Ampliwax Gem-100, Perkin-Elmer) was added and the tubes heated to 77°C for 1 minute and cooled to room temperature to form a wax barrier. 2.5 ul of 10X PCR buffer, 5.0 ul of 50% glycerol/1 mM cresol red, 0.2 ul Taq polymerase (AmpliTaq 5U/ul, Perkin-Elmer) and 15.3 ul of sterile water was added to the tube along with 2.0 ul of DNA from the fungal whole cell lysate described above. 50 cycles of thermal cycling was carried out at 94°C - 30 sec, 40°C - 1 min, 72°C - 2 min. The amplified DNA was electrophoresed and purified from a low melt agarose gel by tris buffered phenol pH 8.0, phenol/chloroform/isoamyl alcohol (25:24:1 by vol.) and 3 ether extractions, followed by isopropanol precipitation and 70% ethanol wash.

We completely sequenced both strands of DNA amplified from the organisms listed above. All sequencing was carried out on an Applied Biosystems 373A sequencer. Every nucleotide in the sequences generated was verified and confirmed by examining the complementary nucleotide from the second strand sequence. We had now created a novel database consisting of nucleic acid sequences spanning a variable region of the 28S rDNA from a diverse collection of medically important fungi.

While the complete sequences for *Candida albicans, Cryptococcus neoformans* and *Saccharomyces cerevisiae* 28S genes had previously been published and deposited in GenBank, it was not obvious, nor had it been defined, whether any regions of sequence identity among these three organisms would also be conserved among all fungi of interest. DNA sequences from all the fungi in our novel 28S database had to be analyzed in order to develop "Universal" 28S probes. All sequences were subjected to extensive manipulation to identify optimal relative

allignments in order to identify regions of similarity for use as "Universal" probes. The selected probe sequences had to meet several important criteria besides the condition of being present in 28S genes from most fungal species. Each probe sequence required an appropriate thermal profile, secondary structure and utility in a DNA amplification reaction. These probes were optimized to work for PCR amplification in pure cultures of fungus, as well as in the presence of DNA from multiple sources as in the case of clinical specimens. The probes were also designed to facilitate direct sequencing of the amplified DNA. Our analysis led to the discovery of the oligonucleotide probes listed in (SEQ ID NO:1) and (SEQ ID NO:2). (For their location, see Figure 1.) The successful identification of these two probes ((SEQ ID NO:1) and (SEQ ID NO:2)) completed our first objective to develop nucleic acid probes that would hybridize to, and detect 28S rRNA and rDNA from a majority of fungi (Figure 1 and Table 1). As shown later in this application, the novel sequence information generated by the use of our "Universal" probes allowed us to develop species-specific probes ((SEQ ID NO:3) to (SEQ ID NO:23)) capable of identifying 19 different disease-causing fungi.

Table 1:

Presence of hybridization sites for probes SEQ ID NO: 1 and SEQ ID NO: 2 in 28S nucleic acid sequences.

	SEQ ID NO: 1	SEQ ID NO: 2
Acremonium sp.	+	+
Aspergillus clavatus	+	+
Aspergillus flavus	+	+
Aspergillus fumigatus	+	+
Aspergillus glaucus	+	+
Aspergillus nidulans	+	+
Aspergillus niger	+	+
Aspergillus ochraceus	+	+

Aspergillus terreus	+	+
Aspergillus unguis	+	+
Aspergillus ustus	+	+
Beauvaria sp.	+	+
Bipolaris sp.	+	+
Blastomyces dermatitidis	+	+
Blastoschizomyces sp.	+	+
Candida albicans	+	+
Candida glabrata	+	+
Candida guilliermondii	+	+
Candida kefyr	+	+
Candida krusei	+	+
Candida lusitaniae	+	+
Candida parapsilosis	+	+
Candida tropicalis	+	+
Chrysosporium sp.	+	+
Cladosporium sp.	+	+
Coccidioides immitis	+	+
Cryptococcus laurentii	+	+
Cryptococcus neoformans serotype A	+	+
Cryptococcus neoformans var. *gattii* serotype B	+	+
Cryptococcus terreus	+	+
Curvularia sp.	+	+
Filobasidiella (Cryptococcus) neoformans var *bacillispora* serotype C	+	+
Filobasidiella (Cryptococcus) neoformans var *neoformans* serotype D	+	+
Filobasidium capsuligenum	+	+
Filobasidium uniguttulatum	+	+
Fusarium sp.	+	+
Geotrichum sp.	+	+
Histoplasma capsulatum	+	+
Malbranchea sp.	+	+
Mucor sp.	+	+
Paecilomyces sp.	+	+
Penicillium sp.	+	+
Pseudallescheria boydii	+	+
Rhizopus sp.	+	+

Saccharomyces cerevisiae	+	+
Scopulariopsis brevicaulis	+	+
Scopulariopsis brumptii	+	+
Sporothrix schenckii	+	+
Trichosporon beigelii	+	+
Human	-	+

Probes SEQ ID NO: 1 and SEQ ID NO: 2 were used to successfully amplify (Table 2) and sequence DNA (Figure 2) spanning this variable region from the following 49 organisms: *Acremonium* sp., *Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Aspergillus unguis, Aspergillus ustus, Beauvaria* sp., *Bipolaris* sp., *Blastomyces dermatitidis, Blastoschizomyces* sp., *Candida albicans, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Chrysosporium* sp., *Cladosporium* sp., *Coccidioides immitis, Cryptococcus neoformans* serotype A, *Cryptococcus neoformans* var. *gattii* serotype B, *Cryptococcus terreus, Cryptococcus laurentii, Curvularia* sp., *Filobasidiella (Cryptococcus) neoformans* var *bacillispora* serotype C, *Filobasidiella (Cryptococcus) neoformans* var *neoformans* serotype D, *Filobasidium capsuligenum, Filobasidium uniguttulatum, Fusarium* sp., *Geotrichum* sp., *Histoplasma capsulatum, Malbranchea* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium* sp., *Pseudallescheria boydii, Rhizopus* sp., *Saccharomyces cerevisiae, Scopulariopsis brevicaulis, Scopulariopsis brumptii, Sporothrix schenkii and Trichosporon beigelii.* This list contains all 4 serotypes (A, B, C and D) of *Cryptococcus neoformans.* This sequence information generated by the use of probes SEQ ID NO: 1 and SEQ ID NO: 2 expanded the size of our database consisting of fungal 28S sequences. All amplified DNA was sequenced across both strands from a minimum of two different isolates of each organism to ensure accuracy of the data generated.

Table 2:

Polymerase chain reaction amplification of 28S rDNA with probes SEQ ID NO: 1 and

SEQ ID NO: 2.

	PCR with SEQ ID NO: 1 & NO: 2
Acremonium sp.	+
Aspergillus clavatus	+
Aspergillus flavus	+
Aspergillus fumigatus	+
Aspergillus glaucus	+
Aspergillus nidulans	+
Aspergillus niger	+
Aspergillus ochraceus	+
Aspergillus terreus	+
Aspergillus unguis	+
Aspergillus ustus	+
Beauvaria sp.	+
Bipolaris sp.	+
Blastomyces dermatitidis	+
Blastoschizomyces sp.	+
Candida albicans	+
Candida glabrata	+
Candida guilliermondii	+
Candida kefyr	+
Candida krusei	+
Candida lusitaniae	+
Candida parapsilosis	+
Candida tropicalis	+
Chrysosporium sp.	+
Cladosporium sp.	+
Coccidioides immitis	+
Cryptococcus laurentii	+
Cryptococcus neoformans serotype A	+
Cryptococcus neoformans var. *gattii* serotype B	+
Cryptococcus terreus	+

Curvularia sp.	+
Filobasidiella (Cryptococcus) neoformans var *bacillispora* serotype C	+
Filobasidiella (Cryptococcus) neoformans var *neoformans* serotype D	+
Filobasidium capsuligenum	+
Filobasidium uniguttulatum	+
Fusarium sp.	+
Geotrichum sp.	+
Histoplasma capsulatum	+
Malbranchea sp.	+
Mucor sp.	+
Paecilomyces sp.	+
Penicillium sp.	+
Pseudallescheria boydii	+
Rhizopus sp.	+
Saccharomyces cerevisiae	+
Scopulariopsis brevicaulis	+
Scopulariopsis brumptii	+
Sporothrix schenckii	+
Trichosporon beigelii	+
Human	-

This list of fungi sequenced by us represents organisms responsible for most cases of subcutaneous and deep mycotic infections in humans and also includes saprophytes (non-pathogenic fungi) commonly encountered in clinical isolates. Since the two probes (SEQ ID NO: 1 and SEQ ID NO: 2) hybridize to 28S rDNA from all the fungi listed above, they are capable of diagnosing the presence of a majority of fungi that are likely to be present in a clinical specimen. They are believed to be primers for universally detecting fungi.

Probes listed in SEQ ID NO: 1 and SEQ ID NO: 2 were also checked for their potential ability to hybridize to, and amplify (in a polymerase chain reaction) 23S sequences from bacteria by searching for hybridization sites among the 539

bacterial 23S genes listed in GenBank. Bacterial 23S rDNAs do not have suitable hybridization sites for SEQ ID NO: 1 and SEQ ID NO: 2 and these two probes should not be able to amplify bacterial DNA under stringent conditions.

Our second objective was to develop species specific probes, which under hybridization conditions, would detect *Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, Aspergillus flavus, Aspergillus glaucus, Aspergillus niger, Aspergillus terreus, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Pseudallescheria boydii,* and *Sporothrix schenckii.* We used our database of fungal 28S nucleic acid sequences to create a multiple sequence alignment of all the organisms that we had sequenced. Every individual sequence was subjected to intensive comparison with all other sequences in our database in order to discover unique regions of sequence that would be present only in the fungus of interest, and would be absent in all other fungi. When unique stretches of sequence were identified, these were further analyzed for thermal profile and secondary structure. Each probe constructed by us will, under conditions of hybridization, specifically hybridize to and detect, nucleic acid sequence from the unique region of only one specific target fungus. Those versed in the art will recognize that specification of a single-stranded DNA sequence implies the utility of the complementary DNA sequence, as well as the two equivalent RNA sequences. Furthermore, sequences incorporating modification of any of the moieties comprising the nucleic acid (i.e., the base, the sugar or the backbone) are functional equivalents of the sequence. It should also be recognized that these additional sequences can potentially serve as probes or primers. Finally, those versed in the art recognize that comparisons of extensive DNA sequences provides enough variability and uniqueness to speciate organisms (Figure 2).

The nucleic acid sequences for these species specific synthetic probes are listed in SEQ ID NO: 3 to SEQ ID NO: 23. There are two probes specific for *Cryptococcus neoformans*, two probes specific for *Sporothrix schenckii*, and one probe each for *Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Histoplasma capsulatum, Aspergillus flavus, Aspergillus glaucus, Aspergillus niger, Aspergillus terreus, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis* and *Pseudallescheria boydii* 28S rRNA and rDNA. (See Tables 3 - 6 and further discussion below.)

All species specific probes developed by us are novel and to the best of our knowledge have not been reported in the literature. While all 28S genes sequenced by us had several regions that were different among the various species analyzed, the regions that would function best as species specific probes under conditions of hybridization were not obvious. Extensive analysis of each 28S sequence yielded several potential probe sites. These were studied in detail to enable the selection of optimal unique sites for each probe, based on the need to obtain optimal hybridization characteristics under the test conditions. The highly specific hybridization characteristics of all probe sequences developed by us were then validated by experimental results. The prior existence in GenBank of sequences for *Candida albicans* and only one serotype of *Cryptococcus neoformans* 28S genes was in itself not sufficient to enable even an individual versed in this field to develop specific probes for either of these two organisms. We had to obtain novel 28S sequence from *Candida albicans, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Cryptococcus neoformans* serotype A, *Cryptococcus neoformans* var. *gattii* serotype B, *Cryptococcus terreus, Cryptococcus laurentii, Filobasidiella (Cryptococcus) neoformans* var *bacillispora* serotype C, *Filobasidiella (Cryptococcus) neoformans* var *neoformans* serotype D, *Filobasidium*

capsuligenum and Filobasidium uniguttulatum before we were able to identify potential regions for the development of species specific probes for these two fungal organisms that would not cross react with the others listed above.

Our modification of the Chomczynski technique (see Example 2, below) allows us to obtain DNA from any clinical specimen, irrespective of source (see Table 8 for a variety of clinical specimens tested), within a 3 hour period. The PCR amplification and subsequent probing can be accomplished with ease within a 24 hour period. The final identification is therefore possible in a day as opposed to several days or weeks required by traditional methods. This speed and sensitivity of diagnosis can make a difference between life and death in debilitated patients battling fungal diseases of undetermined cause. Rapid diagnosis will allow physicians to immediately direct their therapy towards curing the identified causative fungus, rather than wait for days or weeks while the patient succumbs to an unknown fungus.

Our probes have the ability to pick out the correct target organism even in a mixed fungal infection because of their high level of specificity. The methods of Hopfer et. al. and Maiwald et. al., do not allow identification of individual species in a mixed fungal infection because restriction fragment length polymorphism results are nearly impossible to interpret when multiple organisms contribute to the restriction fragments. Their method can therefore only be used on a pure culture, and this also does not save any diagnostic time, because the fungus first has to be grown in culture.

The probes developed by us allow rapid species identification of a large number of pathogenic fungi by using multiple probes against only one PCR amplified fragment of DNA. Coupled with our modified DNA extraction technique and our ability to accurately diagnose in the case of mixed organisms, this strategy

can provide the greatest amount of diagnostic information in the shortest amount of time. This diagnostic strategy is also amenable to automation, which can result in even greater savings in time, money and effort.

The sequences and the complement of the sequences claimed in this disclosure, along with any modifications to these sequences, may potentially be utilized in assays for the identification of fungi based on several existing methodologies, as well as future improvements and alterations of this technology. These techniques include, but are not limited to, assays based on hybridization, ligation, polymerization, depolymerization, sequencing, chemical degradation, enzymatic digestion, electrophoresis, chromatography and amplification. Furthermore, all such variations ultimately are based in some selection or amplification process, some ligand or some nucleic acid moiety that recognizes or utilizes the sequences (SEQ ID NO: 1) to (SEQ ID NO:23) claimed in this application. Such variations include but are not limited to use of a variety of linear or exponential target amplification schemes, such as, any of the myriad forms of PCR, the ligase chain reaction, Q-beta repliase, etc.; direct detection of species-specific nucleic acid purified or extracted from pure fungal culture using a probe selected from the group (SEQ ID NO: 3) to (SEQ ID NO: 23); use of the complementary DNA forms of (SEQ ID NO:1) to (SEQ ID NO:23); use of the RNA forms of these sequences and their complements; and use of derivatives of these DNA or RNA sequences by the addition of one or more reporter moieties from a variety of labels including nucleic acid sequences, proteins, signal generating ligands such as acridinium esters, and/or paramagnetic particles. These techniques may be utilized with DNA, RNA or modified derivatives used as either the target or the detection molecule.

In addition to the 23 sequences SEQ ID NO: 1 to SEQ ID NO: 23, we also describe an additional 51 sequences SEQ ID NO: 24 to SEQ ID NO: 74. These 51

sequences are inclusive of SEQ ID NO: 3 to SEQ ID NO: 23 and are shown as a multiple sequence alignment (Figure 2) with coordinate 1 corresponding to base # 431 of a reference *S. cerevisiae* 28S rRNA gene. (The numbers are comparable to the primary sequence of *S. cerevisiae* 28S rRNA gene. Genbank accession number: J01355). These sequences were obtained by amplifying and sequencing 28S rDNA from various fungi with primers SEQ ID NO: 1 and SEQ ID NO: 2. (SEQ ID NO: 1 corresponds to coordinates 403-422 and the SEQ ID NO: 2 corresponds to coordinates 645-662 of the reference *S. cerevisiae* gene).

An analysis of these aligned sequences enabled us to develop the species specific probes SEQ ID NO: 3 to SEQ ID NO: 23, and sites for these probes are shown underlined. These 51 aligned sequences contain sufficient variability, to enable a person versed in this art, to develop additional species specific hybridization probes in the 10-50 nucleotide length. Similarly, longer species specific hybridization probes encompassing the entire 200+ nucleotide length can also be envisioned. Species identification may also be accomplished by direct DNA sequence determination of any DNA amplified with primers SEQ ID NO: 1 and SEQ ID NO: 2. If the derived sequence matches approximately 98% or more of any sequence in SEQ ID NO: 24 to SEQ ID NO: 74, then the identity of the organism can be ascertained. Additionally, we recognize that parts of SEQ ID NO: 24 to SEQ ID NO: 74 may be specific for groups of fungi arranged phylogenetically at the level of genus or higher. SEQ ID NO: 24 to SEQ ID NO: 74, their complements, along with any modification to these sequences may also potentially be utilized in assays for the identification of fungi based on existing methodologies and future technologies as noted above for SEQ ID NO: 1 to SEQ ID NO: 23.

Legend to figure 2:

The multiple sequence alignment shows the sequence of 28S ribosomal RNA genes amplified with primers SEQ ID NO: 1 and SEQ ID NO: 2. 21 species specific probes (SEQ ID NO: 3 to SEQ ID NO: 23) are shown underlined. Minor sequence variation among two isolate of the same organism are represented by the appropriate code (see key below). Major differences among *Rhizopus* species are depicted by including 3 separate *Rhizopus* sequences in the alignment. (The organisms in this figure are listed according to their sequence relatedness.)

Key to symbols:

(.) gap in sequence to facilitate alignment
(R) A or G
(W) A or T
(Y) T or C
(M) A or C
(K) T or G
(S) G or C
(B) T,G or C

Acremo	*Acremonium* species
A_clav	*Aspergillus clavatus*
A_flav	*Aspergillus flavus*
A_fumi	*Aspergillus fumigatus*
A_glau	*Aspergillus glaucus*
A_nidu	*Aspergillus nidulans*
A_nige	*Aspergillus niger*
A_ochr	*Aspergillus ochraceus*

A_terr	*Aspergillus terreus*
A_ungu	*Aspergillus unguis*
A_ustu	*Aspergillus ustus*
Beauve	*Beauveria* species
Bipola	*Bipolaris* species
Blasch	*Blastoschizomyces* species
B_derm	*Blastomyces dermatitidis*
Chryso	*Chrysosporium* species
Clados	*Cladosporium* species
Curvul	*Curvularia* species
C_albi	*Candida albicans*
C_glab	*Candida glabrata*
C_guil	*Candida guilliermondii*
C_immi	*Coccidioides immitis*
C_kefy	*Candida kefyr*
C_krus	*Candida krusei*
C_laur	*Cryptococcus laurentii*
C_lusi	*Candida lusitaniae*
C_neob	*Cryptococcus neoformans* var *gattii* serotype B
C_neof	*Cryptococcus neoformans* serotype A
C_para	*Candida parapsilosis*
C_terr	*Cryptococcus terreus*
C_trop	*Candida tropicalis*
Fusari	*Fusarium* species
F_caps	*Filobasidium capsuligenum*
F_neoc	*Filobasidiella* (Cryptococcus) *neoformans* var *bacillispora* serotype C
F_neod	*Filobasidiella* (Cryptococcus) *neoformans* var *neoformans* serotype D

F_unig	*Filobasidium uniguttulatum*
Geotri	*Geotrichum* species
H_caps	*Histoplasma capsulatum*
Malbra	*Malbranchea* species
Mucor_	*Mucor* species
Paecil	*Paecilomyces* species
Penici	*Penicillium* species
P_boyd	*Pseudallescheria boydii*
Rhizo1	*Rhizopus* species isolate #1
Rhizo2	*Rhizopus* species isolate #2
Rhizo3	*Rhizopus* species isolate #3
Sporot	*Sporothrix schenkii*
S_brev	*Scopulariopsis brevicaulis*
S_brum	*Scopulariopsis brumpti*
S_cere	*Saccharomyces cerevisiae*
T_beig	*Trichosporon beigelii*

Further variations of the invention that utilize any of the named sequences will be apparent to those with ordinary skill in the art. The following examples illustrate various aspects of the invention but are not intended to limit its usefulness.

EXAMPLE 1. Testing probes SEQ ID NO: 3 to SEQ ID NO: 23 for hybridization specificity.

Probes listed in SEQ ID NO: 3 to SEQ ID NO: 23 were tested for specificity against their target organisms. Probe SEQ ID NO: 5 for *Candida albicans* was the first one tested against a panel of fungi taken from the Mayo Clinic collection. 28S rDNA from *Acremonium* sp., *Aspergillus clavatus, Aspergillus flavus, Aspergillus*

fumigatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Aspergillus unguis, Aspergillus ustus, Aspergillus sp., *Beauvaria* sp., *Bipolaris* sp., *Blastomyces dermatitidis, Candida albicans, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Chrysosporium* sp., *Cladosporium* sp., *Coccidioides immitis, Cryptococcus neoformans* serotype A, *Curvularia* sp., *Fusarium* sp., *Geotrichum* sp., *Histoplasma capsulatum, Mucor* sp., *Penicillium* sp., *Pseudallescheria boydii, Rhizopus* sp., *Saccharomyces cerevisiae, Scopulariopsis brevicaulis, Sporothrix schenkii and Trichosporon beigelii* was amplified in a polymerase chain reaction using oligonucleotide probes SEQ ID NO: 1 and SEQ ID NO: 2. All PCR amplifications were carried out as hot-start reactions in a 50 ul reaction volume using Perkin-Elmer (Norwalk, CT) 0.5 ml thin-wall polypropylene tubes and a Perkin-Elmer thermal cycler. Reagents added to the tube initially were 2.5 ul of 10X PCR buffer (100 mM tris pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 5.0 ul of 50% glycerol/1 mM cresol red, 8.0 ul of dNTP mix (1.25 mM each of dATP, dGTP, dTTP and dCTP), 11 picomoles of each nucleic acid primer and sterile water to make up a volume of 25 ul. A wax bead (Ampliwax Gem=100, Perkin-Elmer) was added and the tubes heated to 77°C for 30 seconds and cooled to room temperature to form a wax barrier. 2.5 ul of 10X PCR buffer, 5.0 ul of 50% glycerol/1 mM cresol red, 0.2 ul Taq polymerase (AmpliTaq 5U/ul, Perkin-Elmer) and 15.3 ul of sterile water was added to the tube along with 2.0 ul of DNA from the fungal whole cell boiled lysate described above. 50 cycles of thermal cycling was carried out at 94°C - 30 sec, 50°C - 1 min, 72°C - 2 min. Five microliters of polymerase chain reaction mix from each sample was run on a 5% polyacrylamide gel to visually confirm the successful amplification of 28S rDNA from each fungus listed above. 40 ul of the remaining amplified 28S rDNA was denatured in 1 N NaOH, and half of this denatured rDNA was slot blotted on to a positively charged polysulphone based membrane equilibrated in 0.5 N NaOH. The membrane was air dried for 15 minutes and baked in a vacuum oven at 80°C for 30

minutes. Amplified rDNA from each species was now bound and immobilized at a separate spot on the membrane. The free binding sites on the membrane were blocked by incubating the membrane for 3 hours at 40°C in hybridization buffer (100 ml of hybridization buffer was made using 1g non-fat milk powder, 6g NaH_2PO_4, 7g SDS, 200 ul 0.5M EDTA and adjusted to pH 7.2 with NaOH). The specific probe for *Candida albicans* (SEQ ID NO: 5) was end-labeled with radioactive phosphorus using ^{32}P ATP and T4 polynucleotide kinase. 50 picomoles of this probe was added to 70 milliliters of hybridization buffer and the membrane was probed at 40°C overnight. The membrane was washed in hybridization buffer at 40°C for 15 minutes followed by a wash in 2X SSC at 40°C for 15 minutes. The membrane was then exposed on x-ray film for at least 1 hour. The oligonucleotide probe SEQ ID NO: 5 only hybridized to amplified 28S rDNA from *Candida albicans* (see Table 3) Under these hybridization conditions, probe SEQ ID NO: 5 is extremely specific for *Candida albicans*. The sequence of oligonucleotide probe SEQ ID NO: 5 differs from the sequences of other species of *Candida* by as few as 1 or 2 bases, but these mismatches are sufficient to prevent stable hybrids from forming with the other *Candida* species.

Probes SEQ ID NO: 3 to SEQ ID NO: 23 were tested for specificity, as described above for the *Candida albicans* probe SEQ ID NO: 5, against the same panel of fungi listed in the preceding paragraph. The positively charged polysulphone based membrane probed with *Candida albicans* probe SEQ ID NO: 5 was washed in 0.5 N NaOH at 40°C for 10 minutes to remove all bound *Candida albicans* probe. The membrane was sequentially probed with all probes listed in SEQ ID NO: 3 to SEQ ID NO: 23. For each subsequently tested probe, the membrane was blocked for at least 30 minutes, probe hybridization was carried out at 40-42°C for at least 3 hours, and post-hybridization washes were done in 2X SSC for 20 minutes. The membrane was stripped between probings by washing in 0.5 to 1.0 N NaOH at 40-42°C. Results are listed in Tables 3 to 6.

As shown in Tables 3 to 6, each probe listed in SEQ ID NO: 3 to SEQ ID NO: 23 specifically hybridizes to only one target fungal 28S nucleic acid sequence. This specificity is essential for identifying a given species of fungus in clinical specimens containing mixed fungal organisms with a high level of reliability. The 39 organisms listed in these Tables represent a majority of organisms that are commonly isolated from clinical samples. While we have developed 21 species specific probes (SEQ ID NO: 3 to SEQ ID NO: 23) that identify a total of 19 individual organisms, the additional organisms listed in the test panel were used to ensure that our probes did not have any cross-reactivity with other fungi likely to be present in a clinical specimen. The ability to accurately and reliably diagnose, and identify to a species level, this large a number of pathogens is unmatched by any other report. The fact that we can achieve this by probing DNA amplified by a single pair of "Universal" probes (SEQ ID NO: 1 and SEQ ID NO: 2) is highly advantageous as it saves time, money and effort by providing the ability to test a single amplified target with 21 different probes (SEQ ID NO: 3 to SEQ ID NO: 23).

A GenBank search was carried out with all probes listed in SEQ ID NO: 3 to SEQ ID NO: 23 in order to determine whether similar gene sequences were present in the database. 28S sequences for *Candida albicans* and one serotype of *Cryptococcus neoformans* are already present in GenBank, and as expected, the probes for *Candida albicans and Cryptococcus neoformans* correctly identified the 28S sequences from these two organsims. Ten other probes also matched DNA sequences from a variety of genes not related to the 28S gene (Table 7). This was expected because short stretches of sequence identity can often be found for any query sequence in unrelated genes from the same or a different organism. This observation is known to those versed in this art. In all cases, sequences that matched a probe sequence were not located within the 28S rRNA genes. Our probes are used to analyze 28S DNA that has been previously amplified in a polymerase chain reaction with our probes SEQ ID NO: 1 and SEQ ID NO: 2. Under stringent

conditions, these two probes only amplify DNA from fungal 28S rRNA genes. Therefore no amplified DNA from the non-28S genes listed in Table 7 will be available for the hybridization of probes SEQ ID NO: 3 to SEQ ID NO: 23. The presence of related sequences in non-28S, unamplified genes will not be detected and will, thus, not have any effect on the sensitivity or the specificity of our detection and identification strategy.

Table 3:

Detection of species specific 28S sequence with probes SEQ ID NO: 3 to SEQ ID NO: 8

FUNGUS	SEQ ID: 3	SEQ ID: 4	SEQ ID: 5	SEQ ID: 6	SEQ ID: 7	SEQ ID: 8
Acremonium sp.	-	-	-	-	-	-
Aspergillus clavatus	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-
Aspergillus fumigatus	+	-	-	-	-	-
Aspergillus glaucus	-	-	-	-	-	-
Aspergillus nidulans	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-
Aspergillus ochraceus	-	-	-	-	-	-
Aspergillus terreus	-	-	-	-	-	-
Aspergillus unguis	-	-	-	-	-	-
Aspergillus ustus	-	-	-	-	-	-
Aspergillus sp.	-	-	-	-	-	-
Beauvaria sp.	-	-	-	-	-	-
Bipolaris sp.	-	-	-	-	-	-
Blastomyces dermatitidis	-	+	-	-	-	-
Candida albicans	-	-	+	-	-	-
Candida glabrata	-	-	-	-	-	-
Candida guilliermondii	-	-	-	-	-	-
Candida kefyr	-	-	-	-	-	-
Candida krusei	-	-	-	-	-	-
Candida lusitaniae	-	-	-	-	-	-
Candida parapsilosis	-	-	-	-	-	-
Candida tropicalis	-	-	-	-	-	-
Chrysosporium sp.	-	-	-	-	-	-
Cladosporium sp.	-	-	-	-	-	-
Coccidioides immitis	-	-	-	+	-	-
Cryptococcus neoformans	-	-	-	-	+	+
Curvularia sp.	-	-	-	-	-	-
Fusarium sp.	-	-	-	-	-	-
Geotrichum sp.	-	-	-	-	-	-
Histoplasma capsulatum	-	-	-	-	-	-
Mucor sp.	-	-	-	-	-	-
Penicillium sp.	-	-	-	-	-	-
Pseudallescheria boydii	-	-	-	-	-	-
Rhizopus sp.	-	-	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-	-	-
Scopulariopsis brevicaulis	-	-	-	-	-	-
Sporothrix schenckii	-	-	-	-	-	-
Trichosporon beigelii	-	-	-	-	-	-

+	Positive
-	Negative after 20 minute wash in 2X SSC

Table 4:

Detection of species specific 28S sequence with probes SEQ ID NO: 9 to SEQ ID NO: 14

FUNGUS	SEQ ID: 9	SEQ ID: 10	SEQ ID: 11	SEQ ID: 12	SEQ ID: 13	SEQ ID: 14
Acremonium sp.	-	-	-	-	-	-
Aspergillus clavatus	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-
Aspergillus fumigatus	-	-	-	-	-	-
Aspergillus glaucus	-	+	-	-	-	-
Aspergillus nidulans	-	-	-	-	-	-
Aspergillus niger	-	-	+	-	-	-
Aspergillus ochraceus	-	-	-	-	-	-
Aspergillus terreus	-	-	-	+	-	-
Aspergillus unguis	-	-	-	-	-	-
Aspergillus ustus	-	-	-	-	-	-
Aspergillus sp.	-	-	-	-	-	-
Beauvaria sp.	-	-	-	-	-	-
Bipolaris sp.	-	-	-	-	-	-
Blastomyces dermatitidis	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	-
Candida glabrata	-	-	-	-	+	-
Candida guilliermondii	-	-	-	-	-	+
Candida kefyr	-	-	-	-	-	-
Candida krusei	-	-	-	-	-	-
Candida lusitaniae	-	-	-	-	-	-
Candida parapsilosis	-	-	-	-	-	-
Candida tropicalis	-	-	-	-	-	-
Chrysosporium sp.	-	-	-	-	-	-
Cladosporium sp.	-	-	-	-	-	-
Coccidioides immitis	-	-	-	-	-	-
Cryptococcus neoformans	-	-	-	-	-	-
Curvularia sp.	-	-	-	-	-	-
Fusarium sp.	-	-	-	-	-	-
Geotrichum sp.	-	-	-	-	-	-
Histoplasma capsulatum	+	-	-	-	-	-
Mucor sp.	-	-	-	-	-	-
Penicillium sp.	-	-	-	-	-	-
Pseudallescheria boydii	-	-	-	-	-	-
Rhizopus sp.	-	-	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-	-	-
Scopulariopsis brevicaulis	-	-	-	-	-	-
Sporothrix schenckii	-	-	-	-	-	-
Trichosporon beigelii	-	-	-	-	-	-

+	Positive
-	Negative after 20 minute wash in 2X SSC

5 Table 5:

Detection of species specific 28S sequence with probes SEQ ID NO: 15 to SEQ ID NO: 20

FUNGUS	SEQ ID: 15	SEQ ID: 16	SEQ ID: 17	SEQ ID: 18	SEQ ID: 19	SEQ ID: 20
Acremonium sp.	-	-	-	-	-	-
Aspergillus clavatus	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-
Aspergillus fumigatus	-	-	-	-	-	-
Aspergillus glaucus	-	-	-	-	-	-
Aspergillus nidulans	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-
Aspergillus ochraceus	-	-	-	-	-	-
Aspergillus terreus	-	-	-	-	-	-
Aspergillus unguis	-	-	-	-	-	-
Aspergillus ustus	-	-	-	-	-	-
Aspergillus sp.	-	-	-	-	-	-
Beauvaria sp.	-	-	-	-	-	-
Bipolaris sp.	-	-	-	-	-	-
Blastomyces dermatitidis	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	-
Candida glabrata	-	-	-	-	-	-
Candida guilliermondii	-	-	-	-	-	-
Candida kefyr	+	-	-	-	-	-
Candida krusei	-	+	-	-	-	-
Candida lusitaniae	-	-	+	-	-	-
Candida parapsilosis	-	-	-	+	-	-
Candida tropicalis	-	-	-	-	+	-
Chrysosporium sp.	-	-	-	-	-	-
Cladosporium sp.	-	-	-	-	-	-
Coccidioides immitis	-	-	-	-	-	-
Cryptococcus neoformans	-	-	-	-	-	-
Curvularia sp.	-	-	-	-	-	-
Fusarium sp.	-	-	-	-	-	-
Geotrichum sp.	-	-	-	-	-	-
Histoplasma capsulatum	-	-	-	-	-	-
Mucor sp.	-	-	-	-	-	-
Penicillium sp.	-	-	-	-	-	-
Pseudallescheria boydii	-	-	-	-	-	+
Rhizopus sp.	-	-	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-	-	-

Scopulariopsis brevicaulis	-	-	-	-	-	-
Sporothrix schenckii	-	-	-	-	-	-
Trichosporon beigelii	-	-	-	-	-	-

+	Positive
-	Negative after 20 minute wash in 2X SSC

5 Table 6:

Detection of species specific 28S sequence with probes SEQ ID NO: 21 to SEQ ID NO: 23

FUNGUS	SEQ ID: 21	SEQ ID: 22	SEQ ID: 23
Acremonium sp.	-	-	-
Aspergillus clavatus	-	-	-
Aspergillus flavus	+	-	-
Aspergillus fumigatus	-	-	-
Aspergillus glaucus	-	-	-
Aspergillus nidulans	-	-	-
Aspergillus niger	-	-	-
Aspergillus ochraceus	-	-	-
Aspergillus terreus	-	-	-
Aspergillus unguis	-	-	-
Aspergillus ustus	-	-	-
Aspergillus sp.	-	-	-
Beauvaria sp.	-	-	-
Bipolaris sp.	-	-	-
Blastomyces dermatitidis	-	-	-
Candida albicans	-	-	-
Candida glabrata	-	-	-
Candida guilliermondii	-	-	-
Candida kefyr	-	-	-
Candida krusei	-	-	-
Candida lusitaniae	-	-	-
Candida parapsilosis	-	-	-
Candida tropicalis	-	-	-
Chrysosporium sp.	-	-	-
Cladosporium sp.	-	-	-
Coccidioides immitis	-	-	-
Cryptococcus neoformans	-	-	-
Curvularia sp.	-	-	-
Fusarium sp.	-	-	-
Geotrichum sp.	-	-	-
Histoplasma capsulatum	-	-	-
Mucor sp.	-	-	-
Penicillium sp.	-	-	-

Pseudallescheria boydii	-	-	-
Rhizopus sp.	-	-	-
Saccharomyces cerevisiae	-	-	-
Scopulariopsis brevicaulis	-	-	-
Sporothrix schenckii	-	+	+
Trichosporon beigelii	-	-	-

+	Positive
-	Negative after 20 minute wash in 2X SSC

5 Table 7:

GenBank search results listing genes from other organisms having 100% identity to probes SEQ ID NO: 3 to SEQ ID NO: 23

	PROBE SEQ ID NO:	ORGANISM MATCHED	GENE MATCHED* (see note below)	ACCESSION NUMBER
Aspergillus fumigatus	3	-	-	-
Blastomyces dermatitidis	4	*Streptomyces verticillus*	bleomycin acetyl transferase	L26955
	4	*Giardia muris*	upstream of rRNA genes	X65063, S53320
	4	*Aspergillus nidulans*	uric acid-xanthine permease	X71807
	4	*Homo sapiens*	T-cell surface glycoprotein	X16996
	4	*Homo sapiens*	MIC2	M16279, M22557, J03841, M22556
Candida albicans	5	*Candida albicans*	28S rRNA	L28817
Coccidioides immitis	6	-	-	-
Cryptococcus neoformans	7	*Cryptococcus neoformans*	28S rRNA	L14067, L14068,
Cryptococcus neoformans	8	*Cryptococcus neoformans*	28S rRNA	L14067, L14068, L20964
	8	*Escherichia coli*	0111 cld	Z17241
Histoplasma capsulatum	9	-	-	-
Aspergillus glaucus	10	*Pseudomonas denitrificans*	cob genes	M62866
Aspergillus niger	11	-	-	-
Aspergillus terreus	12	Human cytomegalovirus	genome	X17403
	12	*Homo sapiens*	GABA receptor	L08485
Candida glabrata	13	*Homo sapiens*	Class 1 MHC	X03664, X03665
Candida guilliermondii	14	-	-	-
Candida kefyr	15	-	-	-

Candida krusei	16	*Pseudomonas syringae*	penicillin binding protein	L28837
Candida lusitaniae	17	Chicken	AK1	D00251
	17	Mouse	IL10	M84340
Candida parapsilosis	18	*Polytomella agilis*	beta-2 tubulin	M33373
	18	Tobacco chloroplast	genome	Z00044, S54304
	18	*Aedes aegypti*	amylase	L03640
	18	*Homo sapiens*	chromosome 13q14	L14473
Candida tropicalis	19	-	-	-
Pseudallescheria boydii	20	*Drosophila melanogaster*	AcTr66B	X71789
		Cow	actin 2	D12816
Aspergillus flavus	21	-	-	-
Sporothrix schenckii	22	-	-	-
Sporothrix schenckii	23	Sulfate reducing bacteria	FMN binding protein	D21804
	23	Equine herpesvirus 1	genome	M86664

* Note: As discussed earlier in this document, the presence of sequences similar to probes SEQ ID NO:3 to SEQ ID NO: 23 in genes not related to 28S does not have any effect on the specificity or sensitivity of our diagnostic strategy. Our species specific probes are used to analyze 28S DNA that has been previously amplified in a polymerase chain reaction with our probes SEQ ID NO: 1 and SEQ ID NO:2. These two probes will not amplify DNA from any gene other than 28S in column #4 (GENE MATCHED), and therefore no amplified DNA from these non-28S genes will be available for the hybridization of probes SEQ ID NO: 3 to SEQ ID NO: 23.

EXAMPLE 2. Use of method in example 1 to test clinical specimens for specific fungal organisms.

Clinical samples taken from the respiratory and gastrointestinal tract of healthy individuals almost always contain some fungal flora. Most of these fungi are non-pathogenic, but may give false positives on traditional immunochemical diagnostic tests for pathogenic fungi.

We obtained 44 clinical specimens from diverse sources ranging from sputum and incision drainage tubes, to intervertebral disc and lung biopsies. Traditional smear and culture results showed that all 44 specimens contained at least 1 type of fungus. In order to test the efficacy of

our probes, we extracted DNA from all 44 clinical samples and used probes SEQ ID NO: 1 & 2 in a polymerase chain reaction to amplify fungal 28S sequences present in these samples.

DNA was extracted from all clinical samples by our modification of the technique of Chomczynski and Sacchi which originally described the use of acid guanidinium thiocyanate-phenol-chloroform to preferentially extract RNA from cells and tissues. We replaced room temperature cell lysis by boiling lysis, and acid guanidinium thiocyanate-phenol-chloroform extraction by alkaline phenol-guanidine thiocyanate to preferentially extract DNA from cells. 1.5 ml Sarsted (Newton, North Carolina) polypropylene screw cap tubes with o-ring seals were used for the extractions. 200 ul of specimen was added to 500 ul of GPT reagent (6 M guanidine thiocyanate dissolved in 50 mM tris pH 8.3 mixed with an equal volume of phenol buffered in tris pH 8.0). This was mixed by vortexing and immediately placed in a boiling water bath for 15 minutes. The tubes were spun in a microcentrifuge for 5 seconds and 250 ul of chloroform/iso-amyl alcohol (24:1 by volume) was added and mixed by vortexing. The liquid phases were separated by centrifugation for 10 minutes and 450 ul of aqueous (upper) phase was transferred to a fresh tube. The aqueous phase was mixed with 500 ul of 100% isopropanol and placed at -20°C for at least 1 hour. At the end of this period the tubes were centrifuged for 15 minutes and the supernatant removed without disturbing the nucleic acid pellet. The pellet was washed with 500 ul of ice-cold 70% ethanol to remove traces of GPT reagent by gently inverting 2 times and then centrifuged for 5 minutes. The ethanol was removed and the pellet dried in a speed vac for 10 minutes. The pellet was resuspended in 25 ul of sterile deionized water and 5 ul was used in a 50 ul PCR amplification. The PCR was carried out as a hot-start reaction using the thermal cycling conditions for probes SEQ ID NO: 1 and SEQ ID NO: 2 described in example 1. Gel electrophoresis showed that probes SEQ ID NO: 1 and SEQ ID NO: 2 successfully amplified DNA from all 44 specimens.

The amplified DNA from each specimen was transferred to a positively charged polysulphone based membrane. We radioactively labeled our species specific probes SEQ ID

NO: 3, SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7, and sequentially probed the membrane to test for the presence of 28S rDNA from *Aspergillus fumigatus, Candida albicans, Coccidioides immitis* and *Cryptococcus neoformans* respectively. Membrane blocking, probe hybridization and washes were done exactly as described in example 1. The results are shown in Table 8.

No false positives were observed, indicating a specificity of 100% for these 4 probes in the clinical specimens tested. 10 out of 12 culture positive samples for *Aspergillus fumigatus*, and 11 out of 13 samples of *Candida albicans* were identified, indicating a detection sensitivity of about 85% for these two probes. Additionally, two out of two *Coccidioides immitis* and two out of two *Cryptococcus neoformans* were correctly identified (detection sensitivity of 100%). As seen by these results, the probes described in this invention can be used on a diverse variety of clinical specimens with excellent efficacy.

Table 8.

Detection of *Aspergillus fumigatus, Candida albicans, Coccidioides immitis and Cryptococcus neoformans* in clinical specimens using species specific probes.

Specimen type	Smear and culture results	PCR with SEQ ID: 1, 2	SEQ ID: 3	SEQ ID: 5	SEQ ID: 6	SEQ ID: 7
U035 sputum	A. flavus	+	-	-	-	-
U069 pleura	A. fumigatus	+	+	-	-	-
U070 bronchial wash	A. flavus	+	-	-	-	-
M019 bronchial wash	A. fumigatus	+	+	-	-	-
M020 sputum	mixed fungal flora	+	-	+	-	-
X35254 sputum	C. albicans	+	-	+	-	-
M20910 sputum	A. fumigatus	+	+	-	-	-
M055 sputum	C. albicans	+	-	+	-	-
M056 abdominal	mixed fungal flora	+	-	-	-	-
M057 drainage tube	C. albicans	+	-	(-)	-	-
M059 ind. sputum	C. albicans	+	-	+	-	-
M060 ind. sputum	mixed fungal flora	+	-	-	-	-
M083 bronchial wash	C. albicans	+	-	+	-	-
M084 sputum	A. fumigatus	+	(-)	-	-	-

M085 throat	C. albicans	+	-	(-)	-	-
A001 sputum	A. fumigatus	+	(-)	-	-	-
A002 leg	Blastomyces	+	-	-	-	-
A003 leg	Blastomyces	+	-	-	-	-
A005 disc	A. fumigatus	+	+	-	-	-
A037 disc	A. fumigatus	+	+	-	-	-
A039 trachea	C. albicans	+	-	+	-	-
A040 trachea	C. albicans	+	-	+	-	-
A102 empyema	A. fumigatus	+	+	-	-	-
Y004 sputum	C. albicans	+	-	+	-	-
Y016 induced sputum	Coccidioides	+	-	-	+	-
Y028 sputum	Coccidioides	+	-	-	+	-
J003 chest	Aspergillus sp.	+	-	-	-	-
J045 bronchial wash	C. albicans	+	-	+	-	-
J046 ethmoid	yeast	+	-	-	-	-
J047 chest	A. fumigatus	+	+	-	-	-
J048 sputum	C. albicans	+	-	+	-	-
J073 lung	Aspergillus sp.	+	-	-	-	-
J074 lung	A. fumigatus	+	+	-	-	-
U017 lip	A. fumigatus	+	+	-	-	-
U033 sputum	mixed fungal flora	+	-	-	-	-
U071 sputum	C. albicans	+	-	+	-	-
U072 BA lavage	Sporothrix	+	-	-	-	-
U073 knee	Histoplasma	+	-	-	-	-
U074 mandible	Cryptococcus	+	-	-	-	+
U075 CSF	Cryptococcus	+	-	-	-	+
U076 knee	Histoplasma	+	-	-	-	-
U077 soft tissue	Histoplasma	+	-	-	-	-
U051 buccal	A. fumigatus	+	+	-	-	-
Y055 sputum	mixed fungal flora	+	-	-	-	-

+ Positive - Negative (-) Missed

EXAMPLE 3. DNA sequence based identification of unknown fungal organisms.

Another utility of our probes is in the rapid DNA sequence based identification of a pure culture of fungus. Probes SEQ ID NO: 1 and SEQ ID NO: 2 are used in a polymerase chain reaction to amplify 28S rDNA from an unknown fungus. Probes SEQ ID NO: 1 or SEQ ID NO: 2 are then used as sequencing primers to obtain DNA sequence from this amplified 28S DNA belonging to the unknown fungus. This DNA sequence is compared to the fungal 28S DNA sequences in our database, and a sequence match at, or overlapping any one of the probe sequences in SEQ ID NO: 3 to SEQ ID NO: 74 will confirm the identity of the fungus. This technique cannot be used directly on clinical samples, as these usually contain DNA from more than one fungus, and the DNA sequence generated will consist of overlapping sequences of several organisms. This technique has utility in rapidly and reliably identifying colonies of a single fungus on culture plates, clinical specimens, food, pharmaceutical, environmental or other samples containing only one species of fungus.

EXAMPLE 4. Capture and identification of target DNA or RNA

All primers and probes described in this invention disclosure may be labeled with any detectable reporter or signal moiety including, but not limited to radioisotopes, enzymes, antigens, antibodies, chemiluminescent reagents and fluorescent chemicals. Additionally, these probes may be modified without changing the substance of their purpose by terminal addition of nucleotides designed to incorporate restriction sites or other useful sequences. These probes may also be modified by the addition of a capture moiety (including, but not limited to para-magnetic particles, biotin, fluorescein, dioxigenin, antigens, antibodies) or attached to the walls of microtiter trays to assist in the solid phase capture and purification of these probes and any DNA or RNA hybridized to these probes. Fluorescein may be used as a signal moiety as well as a capture moiety, the latter by interacting with an anti-fluorescein antibody.

A typical utility of these modifications would be as follows. Primers SEQ ID NO: 1 and SEQ ID NO: 2 would be utilized to amplify 28S rDNA from a sample, if present, as described previously. Primers would be modified so as to contain a biotin moiety at their 5' ends. A streptavidin solid phase, such as a paramagnetic particle, would be used to separate PCR products, if present, from the reaction mixture. The amplified target may be subsequently hybridized to a third probe ((SEQ ID NO: 3) to (SEQ ID NO: 74) or their complements) attached to a detectable moiety to determine which species of fungus is present in the given sample. Multiple probes, each labeled with a different detectable moiety may be used at one time to analyze the amplified target.

Alternatively, Primers SEQ ID NO: 1 and SEQ ID NO: 2 would be utilized to amplify 28S rDNA from a sample, if present, as above. In a separate reaction, individually, either SEQ ID NO: 1 or SEQ ID NO: 2 would be modified by attachment to a solid phase capture moiety, such as a paramagnetic particle, and SEQ ID NO: 3 to SEQ ID NO: 74 (or their complements) would be modified by addition of a detectable moiety. Alternately, in the amplicon, any sequences delimited by SEQ ID NO: 1 and SEQ ID NO: 2, including but not limited to SEQ ID NO: 3 to SEQ ID NO: 74, may be used in the design of a capture probe. One of the probes attached to a solid phase (SEQ ID NO: 1 and SEQ ID NO: 2) or any other appropriately designed sequences and one of the probes modified by attachment to a detectable moiety (SEQ ID NO: 3 to SEQ ID NO: 74 or their complements) would be hybridized together, in solution, to products of the PCR, if they had been generated. The hybrids, if present, would be captured from the solution, and analyzed by a method appropriate to the detection moiety. Detection of the hybridized probe would indicate which species of fungus was present in the given sample. Multiple probes, each labeled with a different detectable moiety may be used at one time to analyze the amplified target.

EXAMPLE 5. Species-specific amplification of fungal DNA

Another utility of the probes described in this invention is their usage as primers in the direct detection of a specific fungal species by virtue of a nucleic acid amplification reaction. In this embodiment, one primer is a universal one, such as (SEQ ID NO:1) or (SEQ ID NO:2), and the other is a species-specific primer selected from the group consisting of (SEQ ID NO:3) to (SEQ ID NO: 23) or the complements thereof. One variation of this approach is the substitution of (SEQ ID NO:1) or (SEQ ID NO:2) with any functional sequence located in proximity to the species-specific primer. Another variation of this approach is the selection of any appropriate species specific primer pair from SEQ ID NO: 24 to SEQ ID NO: 74.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) Sandhu, Gurpreet S.

(B) Kline, Bruce C.

(ii) TITLE OF INVENTION:

Nucleic Acid Probes for the Detection and Identification of Fungi

(iii) NUMBER OF SEQUENCES: 23

(iv) CORRESPONDENCE ADDRESS:

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(B) STREET: 63 North Street

(C) CITY: Medfield

(D) STATE: Massachusetts

(E) COUNTRY: USA

(F) ZIP: 02052

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette 3.5 inch, 1.44 Mb storage

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: MS-DOS 6.2

(D) SOFTWARE: Word 6.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(viii) ATTORNEY INFORMATION:

(A) NAME: Morgenstern, Arthur S.

(B) REGISTRATION NUMBER: 28,244

(C) DOCKET NUMBER: CCD-180

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 508 359-3836

(B) TELEFAX: 508 359-3885

(2) INFORMATION FOR SEQ ID NO 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for fungal organisms

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTGAAATTGT TGAAAGGGAA 20

(3) INFORMATION FOR SEQ ID NO 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for fungal organisms

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GACTCCTTGG TCCGTGTT 18

(4) INFORMATION FOR SEQ ID NO 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Aspergillus fumigatus*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTCGGAATGT ATCA 14

(5) INFORMATION FOR SEQ ID NO 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Blastomyces dermatitidis*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ACTCCCCCAC GGG 13

(6) INFORMATION FOR SEQ ID NO 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Candida albicans*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCTCTGACGA TGCT 14

(7) INFORMATION FOR SEQ ID NO 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Coccidioides immitis*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TCTGGCGGTT GGTT 14

(8) INFORMATION FOR SEQ ID NO 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Cryptococcus neoformans*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTCCTGTCGC ATAC 14

(9) INFORMATION FOR SEQ ID NO 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Cryptococcus neoformans*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AGTTCTGATC GGTG 14

(10) INFORMATION FOR SEQ ID NO 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Histoplasma capsulatum*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CAATCCCCCG CGGC 14

(11) INFORMATION FOR SEQ ID NO 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Aspergillus glaucus*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTGTCATGCG GCCA 14

(12) INFORMATION FOR SEQ ID NO 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Aspergillus niger*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CCCTGGAATG TAGT 14

(13) INFORMATION FOR SEQ ID NO 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Aspergillus terreus*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GCTTCGGCCC GGTG 14

(14) INFORMATION FOR SEQ ID NO 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Candida glabrata*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CTTGGGACTC TCGC 14

(15) INFORMATION FOR SEQ ID NO 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Candida guilliermondii*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

ATATTTTGTG AGCC 14

(16) INFORMATION FOR SEQ ID NO 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Candida kefyr*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TTCGGCTTTC GCTG 14

(17) INFORMATION FOR SEQ ID NO 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Candida krusei*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGGATTGCGC ACCG 14

(18) INFORMATION FOR SEQ ID NO 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Candida lusitaniae*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GCCTCCATCC CTTT 14

(19) INFORMATION FOR SEQ ID NO 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Candida parapsilosis*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

ATAAGTGCAA AGAA 14

(20) INFORMATION FOR SEQ ID NO 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Candida tropicalis*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

AGAATTGCGT TGGA 14

(21) INFORMATION FOR SEQ ID NO 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Pseudallescheria boydii*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GCGATGGGAA TGTG 14

(22) INFORMATION FOR SEQ ID NO 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Aspergillus flavus*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

AGACTCGCCT CCAG 14

(23) INFORMATION FOR SEQ ID NO 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Sporothrix schenckii*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CGGACCACCC GGCG 14

(24) INFORMATION FOR SEQ ID NO 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Nucleic acid probe for *Sporothrix schenckii*

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CGGCGGCATG CCCC 14

(25) INFORMATION FOR SEQ ID NO 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 208

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Acremonium* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GACCAGACTT GGGCTCGGTG AATCATCCGG CGTTCTCGCC GGTGCACTTT
GCCGTCCCAG GCCAGCATCA GTTCGCGCCG GGGGATAAAG GTTTCGGGAA
TGTAGCTCCT TCGGGAGTGT TATAGCCCGT TGCGTAATAC CCTGGCGTGG
ACTGAGGTCC GCGCTCTGCA AGGATGCTGG CGTAATGGTC ATCAGTGACC
CGTCTTGA

(26) INFORMATION FOR SEQ ID NO 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus clavatus* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GACCAGACTC GCTCGCGGGG TTCAGCCGGC ATTCGTGCCG GTGTACTTCC
CCGTGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCCTCCGGAA
TGTATCACCT CTCGGGGTGT CTTATAGCCG GGGGTGCAAT GCGGCCTGCC
TGGACCGAGG AACGCGCTTC GGCTCGGACG CTGGCGTAAT GGTCGTAAAT
GACCCGTCTT GA

(27) INFORMATION FOR SEQ ID NO 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus flavus* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GACCAGACTC GCCTCCAGGG TTCAGCCGGC ATTCGTGCCG GTGTACTTCC
CTGGGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCTCCCGGAA
TGTAGTGCCC TYCGGGGCAC CTTATAGCCG GGAGTGCAAT GCGGCCAGCC
TGGACCGAGG AACGCGCTTC GGCACGGACG CTGGCATAAT GGTCGYAAAC
GACCCGTCTT GA

(28) INFORMATION FOR SEQ ID NO 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus fumigatus* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
GACCAGACTC GCCCGCGGGG TTCAGCCGGC ATTCGTGCCG GTGTACTTCC
CCGTGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCCCTCGGAA
TGTATCACCT CTCGGGGTGT CTTATAGCCG AGGGTGCAAT GCGGCCTGCC
TGGACCGAGG AACGCGCTTC GGCTCGGACG CTGGCGTAAT GGTCGTAAAT
GACCCGTCTT GA
```

(29) INFORMATION FOR SEQ ID NO 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus glaucus* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GACCAGACTC GCTTCCGGGG TTCAGCCGGC TTTCGGGCCG GTGTACTTCC
CCGGGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCCCCTGGAA
TGTAACGCCT CTCGGGGCGC CTTATAGCCA GGGGTGTCAT GCGGCCAGCC
TGGACCGAGG AACGCGCTTC GGCACGGACG CTGGCATAAT GGTCGTAAAC
GACCCGTCTT GA

(30) INFORMATION FOR SEQ ID NO 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 213

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus nidulans* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GACCAGACTC GGCCCCGGGG TTCARCCAGC ACTCGTGCTG GTGTACTTCC
CCGGGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCCCCAGGAA
TGTATCGCCC TCCGGGGTTG TCTTATAGCC TGGGGTGCAA TGCGGCCAGC
CCGGACCGAG GAACGCGCTT CGGCACGGAC GCTGGCGTAA TGGTCGCAAA
CGACCCGTCT TGA

(31) INFORMATION FOR SEQ ID NO 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus niger* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GACCAGACTC GCCCGCGGGG TTCAGCCGGC ATTCGTGCCG GTGTACTTCC
CCGTGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCCCCTGGAA
TGTAGTRCCC TCCGGGGYAC CTTATAGCCA GGGGTGCAAT GCGGCCAGCC
TGGACCGAGG AACGCGCTTC GGCACGGACG CTGGCATAAT GGTCGTAAAC
GACCCGTCTT GA

(32) INFORMATION FOR SEQ ID NO 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus ochraceus* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

```
GACCAGACTC GCCCGCGGGG TTCAGCCGGC ATTCGTGCCG GTGTACTTCC
CCGCGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCCCCCGGAA
TGTAGCACCC TTCGGGGTGC CTTATAGCCG GGGGTGCAAT GCGGCCAGCC
TGGACCGAGG AACGCGCTTC GGCACGGACG CTGGCATAAT GGTCGTAAAC
GACCCGTCTT GA
```

(33) INFORMATION FOR SEQ ID NO 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus terreus* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

AACCAGACTC GCTCGCGGGG TTCAGCCGGG CTTCGGCCCG GTGTACTTCC
CCGCGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCCTCCGGAA
TGTAGCGCCC TTCGGGGCGC CTTATAGCCG GGGGTGCAAT GCGGCCAGCC
TGGACCGAGG AACGCGCTTC GGCACGGACG CTGGCATAAT GGTTGTAAAC
GACCCGTCTT GA

(34) INFORMATION FOR SEQ ID NO 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 213

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus unguis* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GACCAGACTC GGCCTCGGGG TTCAGCCAGC ACTCGTGCTG GTGTACTTCC
CCGGGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCCCCAGGAA
TGTATCACCC TCCGGGGTTG TCTTATAGCC TGGGGTGCAA TGCGGCCAGC
CTGGACCGAG GAACGCGCTT CGGCACGGAC GCTGGCATAA TGGTTGCAAA
CGACCCGTCT TGA

(35) INFORMATION FOR SEQ ID NO 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Aspergillus ustus* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GACCAGACTC GGCCCCGGGG TTCAGCCAGC ACTCGTGCTG GTGTACTTCC
CCGGGGGCGG GCCAGCGTCG GTTTGGGCGG CCGGTCAAAG GCCCCAGGAA
TGTGTCGCCC TCCGGGGCGT CTTATAGCCT GGGGTGCAAT GCGGCCAGCC
CGGACCGAGG AACGCGCTTC GGCACGGACG CTGGCGTAAT GGTCGCAAAC
GACCCGTCTT GA

(36) INFORMATION FOR SEQ ID NO 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 208

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Beauveria* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GACCAGACTT GGGCTTGGTT GATCATCCGG GGTTCTCCCC GGTGCACTCT
TCCGGCCCAG GCCAGCATCA GTTCGCCCTG GGGGACAAAG GCTTCGGGAA
CGTGGCTCTC TCCGGGGAGT GTTATAGCCC GTTGCGTAAT ACCCTGTGGC
GGACTGAGGT TCGCGCATTC GCAAGGATGC TGGCGTAATG GTCATCAGTG
ACCCGTCT

(37) INFORMATION FOR SEQ ID NO 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 213

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Bipolaris* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

AGCCAGACTT GCTTGCAGTT GCTCATCCGG GCTTTTGCCC
GGTGCACTCT TCTGCAGGCA GGCCAGCATC AGTTTGGGCG
GTGGGATAAA GGTCTCTGTC ACGTACCTTC CTTCGGGTTG
GCCATATAGG GGAGACGTCA TACCACCAGC CTGGACTGAG
GTCCGCGCAT CTGCTAGGAT GCTGGCGTAA TGGCTGTAAG
CGGCCCGTCT TGA

(38) INFORMATION FOR SEQ ID NO 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 105

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Blastoschizomyces* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

TGAAATTGTT GAAAGGGAAG GCGATGGTAG GAATAAGAGG CTGCGGTTTG
AAATAATTGT TTTTCGGGCC ACGGTCTCCT GAGCCTGCTT TCGCACCCGT
CTTGA

(39) INFORMATION FOR SEQ ID NO 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 214

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Blastomyces dermatitidis* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GACCAGAGTC GGCCGTGGGG GTTCAGCGGG CATTCGTTGC CCGTGCACTC
CCCCACGGGC GGGCCAGCGT CGGTTTCGAC GGCCGGTCAA AGGCCCCCGG
AATGTGTCGC CTCTCGGGGC GTCTTATAGC CGGGGGTGCA ATGCGGCCAG
TCGGGACCGA GGAACGCGCT TCGGCACGGA CGCTGGCTTA ATGGTCGTAA
GCGACCCGTC TTGA

(40) INFORMATION FOR SEQ ID NO 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 213

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Chrysosporium* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

```
AACCAGACTT GCGCGCGGCC GATCATCCGG TGTTCTCACC GGTGCACTCG
GCCGTGCTCA GGCCAGCATC GGTTTTGGCG GCTGGATAAA GGCCCTAGGA
ATGTGGCTCC TCTCGGGGAG TGTTATAGCC TAGGGTGCAA TGCAGCCTGC
TGGGACCGAG GACCGCGCTT CGGCTAGGAT GCTGGCGTAA TGGTTGTAAG
CGGCCCGTCT TGA
```

(41) INFORMATION FOR SEQ ID NO 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 207

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Cladosporium* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

AACCAGACTT GCTCGCGGTG TTCCGCCGGT CTTCTGACCG GTCTACTCGC
CGCGTTGCAG GCCAGCATCG TCTGGTGCCG CTGGATAAGA CTTGAGGAAT
GTAGCTCCCT CGGGAGTGTT ATAGCCTCTT GTGATGCAGC GAGCGCCGGG
CGAGGTCCGC GCTTCGGCTA GGATGCTGGC GTAATGGTCG TAATCCGCCC
GTCTTGA

(42) INFORMATION FOR SEQ ID NO 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 213

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Curvularia* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

AGCCAGACTT GCTTGCAGTT GCTCATCCGG GCTTTTGCCC GGTGCACTCT
TCTGCAGGCA GGCCAGCATC AGTTTGGGCG GTGGGATAAA GGTCTCTGAC
ACGTTCCTTC CTTCGGGTTG GCCATATAGG GGAGACGTCA TACCACCAGC
CTGGACTGAG GTCCGCGCAT CTGCTAGGAT GCTGGCGTAA TGGCTGTAAG
CGGCCCGTCT TGA

(43) INFORMATION FOR SEQ ID NO 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 213

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Candida albicans* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

GATCAGACTT GGTATTTTGC ATGCTGCTCT CTCGGGGGCG GCCGCTGCGG
TTTACCGGGC CAGCATCGGT TTGGAGCGGC AGGATAATGG CGGAGGAATG
TGGCACGGCT TCTGCTGTGT GTTATAGCCT CTGACGATGC TGCCAGCCTA
GACCGAGGAC TGCGGTTTTT AACCTAGGAT GTTGGCATAA TGATCTTAAG

TCGCCCGTCT TGA

(44) INFORMATION FOR SEQ ID NO 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 223

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Candida glabrata* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GATCAGACAT GGTGTTTTGC GCCCCTTGCC TCTCGTGGGC TTGGGACTCT
CGCAGCTCAC TGGGCCAGCA TCGGTTTTGG CGGCCGGAAA AAACCTAGGG
AATGTGGCTC TGCGCCTCGG TGTAGAGTGT TATAGCCCTG GGGAATACGG
CCAGCCGGGA CCGAGGACTG CGATACTTGT TATCTAGGAT GCTGGCATAA
TGGTTATATG CCGCCCGTCT TGA

(45) INFORMATION FOR SEQ ID NO 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Candida guilliermondii* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GATCAGACTC GATATTTTGT GAGCCTTGCC TTCGTGGCGG GGTGACCCGC
AGCTTATCGG GCCAGCATCG GTTTGGGCGG TAGGATAATG GCGTAGGAAT
GTGACTTTRC TTCGGTGAAG TGTTATAGCC TGCGTTGATG CTGCCTGCCT
AGACCGAGGA CTGCGATTTT ATCAAGGATG CTGGCATAAT GATCCCAAAC
CGCCCGTCTT GA

(46) INFORMATION FOR SEQ ID NO 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 214

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Coccidioides immitis* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

AACCAGACTC GGTCGTGGGG GCTCAGCGGG CATGAGTGCC CGTGTACTCC
CCCATGCTCC GGGCCAGCAT CAGTTCTGGC GGTTGGTTAA AGGCCTCTGG
AATGTATCGT CCTCCGGGAC GTCTTATAGC CAGGGGCGCA ATGCGGCCAG
CCGGGACTGA GGAACGCGCT TCGGCACGGA TGCTGGCATA ATGGTTGTAA
GCGGCCCGTC TTGA

(47) INFORMATION FOR SEQ ID NO 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 187

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Candida kefyr* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

GATCAGACAT GGCGTTTGCT TCGGCTTTCG CTGGGCCAGC ATCAGTTTTA
GCGGTTGGAT AAATCCTCGG GAATGTGGCT CTGCTTCGGT AGAGTGTTAT
AGCCCGTGGG AATACAGCCA GCTGGGACTG AGGATTGCGA CTTTTGTCAA
GGATGCTGGC GTAATGGTTA AATGCCGCCC GTCTTGA

(48) INFORMATION FOR SEQ ID NO 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 213

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Candida krusei* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

```
CGCCCGACAT CGGGATTGCG CACCGCTGCC TCTCGTGGGC GGCGCTCTGG
GCTTTCCCTG GGCCAGCATC GGTTCTTGCT GCAGGAGAAG GGGTTCTGGA
ACGTGGCTCT TCGGAGTGTT ATAGCCAGGG CCAGATGCTG CGTGCGGGGA
CCGAGGACTG CGGCCGTGTA GGTCACGGAT GCTGGCAGAA CGGCGCAACA
CCGCCCGTCT TGA
```

(49) INFORMATION FOR SEQ ID NO 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 236

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Cryptococcus laurentii* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

```
AGTCAGTCGT GTCTGGGAGG CTCAGCCGGT TCTGCCGGTG TATTCCTCTC
AGACGGGTCA ACATCAGTTT TGTCCGACGG ATAATGGCGG CGGGAAAGTA
GCACCTCCGG GTGTGTTATA GCCCGCTGTC GCATACGCCG GATGAGACTG
AGGCATGCAG CTCGCCTTTA TGGCAGGGGT TCGCCCACTT TCGAGCTTAG
GATGTTGACG TAATGGCTTT AAACGACCCG TCTTGA
```

(50) INFORMATION FOR SEQ ID NO 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 173

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Candida lusitaniae* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

AAGCAGACAC GGTTTTACCG GGCCAGCGTC GAAAAGGGGG GAGGAACAAG
AACTCGAGAA TGTGGCGCGC ACCTTCGGGY GCGCGTGTTA TAGCTCGTGT
TGACGCCTCC ATCCCTTTTC GAGGCCTGCG ATTCTAGGAC GCTGGCGTAA
TGGTTGCAAG CCGCCCGTCT TGA

(51) INFORMATION FOR SEQ ID NO 50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 238

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Cryptococcus neoformans* var *gattii* (serotype B) specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

AGTCAGTCGT GTCTATTGGG TTCAGCCAGC TCTGCTGGTG TATTCCCTTT
AGACGGGTCA ACATCAGTTC TGATCGGTGG ATAAGGGCTG GAGGAATGTG
GCACTCTTCG GGGTGTGTTA TAGCCTCCTG TCGCATACAC TGGTTGGGAC
TGAGGAATGC AGCTCGCCTT TATGGCCGGG GTTCGCCCAC GTTCGAGCTT

AGGATGTTGA CAAAATGGCT TTAAACGACC CGTCTTGA

(52) INFORMATION FOR SEQ ID NO 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 238

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Cryptococcus neoformans* (serotype A) specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

AGTCAGTCGT GTCTATTGGG TTCAGCCAGT TCTGCTGGTG TATTCCCTTT
AGACGGGTCA ACATCAGTTC TGATCGGTGG ATAAGGGCTG GGGGAATGTA
GCACTCTTCG GAGTGTGTTA TAGCCTCCTG TCGCATACAC TGGTTGGGAC
TGAGGAATGC AGCTCGCCTT TATGGCCGGG GTTCGCCCAC GTTCGAGCTT
AGGATGTTGA CAAAATGGCT TTAAACGACC CGTCTTGA

(53) INFORMATION FOR SEQ ID NO 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 211

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Candida parapsilosis* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

```
GATCAGACTT GGTATTTTGT ATGTTACTCT CTCGGGGGTG GCCTCTACAG
TTTACCGGGC CAGCATCAGT TTGAGCGGTA GGATAAGTGC AAAGAAATGT
GGCACTGCTT CGGTAGTGTG TTATAGTCTT TGTCGATACT GCCAGCTTAG
ACTGAGGACT GCGGCTTCGG CCTAGGATGT TGGCATAATG ATCTTAAGTC
GCCCGTCTTG A
```

(54) INFORMATION FOR SEQ ID NO 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 238

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Cryptococcus terreus* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

AGTCAGTCAT GTCTATTGGA CTCAGCCGGT TCTGCCGGTG TACTTCCTTT
AGATGGGGTC AACATCAGTT TTGATCGCTG GAAAAGGGCA GGAGGAATGT
AGCACTCTCG GGTGAACTTA TAGCCTTCTG TCGTATACAG TGGTTGGGAC
TGAGGAACGC AGCATGCCTT TATGGCCGGG GTTCGCCCAC GTACATGCTT
AGGATGTTGA CATAATGGCT TTAAACGACC CGTCTTGA

(55) INFORMATION FOR SEQ ID NO 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 211

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Candida tropicalis* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

GATCAGACTT GGTATTTTGT ATGTTACTTC TTCGGGGGTG GCCTCTACAG
TTTATCGGGC CAGCATCAGT TTGGGCGGTA GGAGAATTGC GTTGGAATGT
GGCACGGCTT CGGTTGTGTG TTATAGCCTT CGTCGATACT GCCAGCCTAG
ACTGAGGACT GCGGTTTATA CCTAGGATGT TGGCATAATG ATCTTAAGTC
GCCCGTCTTG A

(56) INFORMATION FOR SEQ ID NO 55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 211

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Fusarium* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

```
GACCAGACTT GGGCTTGGTT AATCATCTGG GGTTCTCYCC AGTGCACTTT
TCCAGTCCAG GCCAGCATCA GTTTTCSCCG GGGGATAAAG RCTTCGGGAA
TGTGGCTCYC YYCGGGGAGT GTTATAGCCC GTTGYGTAAT ACCCTGGBGG
GGACTGAGGT TCGCGCWTCT GCAAGGATGC TGGCGTAATG GTCATCAACG
ACCCGTCTTG A
```

(57) INFORMATION FOR SEQ ID NO 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 238

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Filobasidium capsuligenum* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

AGTCAGTCAT GTCTATTGGA CTCAGCCGGT TCTGCCGGTG TATTTCCTTT
AGATGGGGTC AACATCAGTT TTGACCGTTG GATAAAGGCA GGAAGAATGT
AGCACTCTCG GGTGAACTTA TAGCTTCTTG TCACATACAA TGGTTGGGAC
TGAGGAACGC AGCATGCCTT TATGGCCGGG ATTCGTCCAC GTACATGCTT
AGGATGTTGA CATAATGGCT TTAAACGACC CGTCTTGA

(58) INFORMATION FOR SEQ ID NO 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 238

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Filobasidiella neoformans* var *bacillispora* (serotype C) specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

AGTCAGTCGT GTCTATTGGG TTCAGCCAGC TCTGCTGGTG TATTCCCTTT
AGACGGGTCA ACATCAGTTC TGATCGGTGG ATAAGGGCTG GAGGAATGTG
GCACTCTTCG GGGTGTGTTA TAGCCTCCTG TCGCATACAC TGGTTGGGAC
TGAGGAATGC AGCTCGCCTT TATGGCCGGG GTTCGCCCAC GTTCGAGCTT
AGGATGTTGA CAAAATGGCT TTAAACGACC CGTCTTGA

(59) INFORMATION FOR SEQ ID NO 58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 238

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Filobasidiella neoformans* var *neoformans* (serotype D) specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

AGTCAGTCGT GTCTATTGGG TTCAGCCAGT TCTGCTGGTG TATTCCCTTT

AGACGGGTCA ACATCAGTTC TGATCGGTGG ATAAGGGCTG GAGGAATGTG
GCACTCTTCG GGGTGTGTTA TAGCCTCCTG TCGCATACAC TGGTTGGGAC
TGAGGAATGC AGCTCGCCTT TATGGCCGGG GTTCGCCCAC GTTCGAGCTT
AGGATGTTGA CAAAATGGCT TTAAACGACC CGTCTTGA

(60) INFORMATION FOR SEQ ID NO 59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 236

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Filobasidium uniguttulatum* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

AGTCAGTCGT GCTCAATGGA CTCAGCCGTT CTGCGGTGTA TTTCCATTGG
GTGGGGTCAA CATCAGTTTT GATCGCTGGA TAAAGGCAGG AGGAATGTAG
CACCCCCGGG TGAACTTATA GCCTCTTGTC ACATACAGTG GTTGGGACTG
AGGAACGCAG CATGCCTTTA TGGCCGGGAT TCGTCCACGT ACATGCTTAG
GATGTTGACA TAATGGCTTT AAACGACCCG TCTTGA

(61) INFORMATION FOR SEQ ID NO 60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 204

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Geotrichum* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

AATCAGACTT GGTGCTGTTG TTCAACTRTG TTTCGGCATA GTGTACTCAG
CAGTACTAGG CCAAGGTGGG GTGTTTGGGA GTGAAAAAGA AGTAGGAACG
TAACTCTTCG GAGTGTTATA GCCTACTTTC ATAGCTCCTC AGGCGCCTCA
GGACTGCGCT TCGGCAAGGA CCTTGGCATA ATGATTCTAT ACCGCCCGTC
TTGA

(62) INFORMATION FOR SEQ ID NO 61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 214

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Histoplasma capsulatum* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

GAYCAGAGTC GGCCGYGGGG GTTCAGCGGG CATTCGTTGC CCGTGCAATC
CCCCGCGGCC GGGCCAGCGT CGGTTTCGAC GGCCGGTCAA AGGCCCCCGG
AATGTGTCGC CTCTCGGGGC GTCTTATAGC CGGGGGTGCA ATGCGGCCAG
TCGGGACCGA GGAACGCGCT CCGGCACGGA CGCTGGCTTA ATGGTCGTCA
GCGACCCGTC TTGA

(63) INFORMATION FOR SEQ ID NO 62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 215

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Malbranchea* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

AGACAGACTC GAGCGCGGGG GCTCAGCGGG TATTGTTATG CCCGTGCACT
CCCCCGCGCC CGGGCCAGCA TCAGTTTTGG CGGCCGGTCA AAGGCCCTTG
GAATGTATCG TCCTCCGGGA CGTCTTATAG CCAAGGGTGC AATGCGGCCA
GCCGGGACTG AGGAACGCGC TTCGGCACGG ATGCTGGCGT AATGGCTGTA
AGCGGCCCGT CTTGA

(64) INFORMATION FOR SEQ ID NO 63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 237

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Mucor* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

AGCCAGACTG GTTTGACTGT AATCAACCTA GAATTCGTTC TGGGTGCACT
TGCAGTCTAT ACCTGCCAAC AACAGTTTGA TTTGGAGGAA AAAATTAGTA
GGAATGTAGC CTCTCGAGGT GTTATAGCCT ACTATCATAC TCTGGATTGG
ACTGAGGAAC GCAGCGAATG CCWTTAGGCR AGATTGCTGG GTGCTTTCGC
TAATAAAATGT TAGAATTTCT GCTTCGGGTG GTGCTAA

(65) INFORMATION FOR SEQ ID NO 64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 209

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Paecilomyces* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

GACCAGACTT GGGCCCGGTG GATCATCCAG CGTTCTCGCT GGTGCACTCC
GCCGGGTTCA GGCCAGCATC AGTTCGCCGC GGGGGAAAAA GGCTTCGGGA
ACGTGGCTCC TACGGGAGTG TTATAGCCCG TTGCATAATA CCCTGGGGCG
GACTGAGGTT CGCGCTCCGC AAGGATGCTG GCGTAATGGT CATCAGCGAC
CCGTCTTGA

(66) INFORMATION FOR SEQ ID NO 65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 199

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Penicillium* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

GACCAGACTC GCCCACGGGG TTCAGCCGGC ATTCGTGCCG GTGTACTTCC
CCGCGGGCGG GCCAGCGTCG GTTTGGKCGG CCGGTCAAAG GCCCTCGGAA
TRTAACGCCC CCCGGGGCGT CTTATAGCCG AGGGTGCCAT GCGGCCAGCM
CAGACCGAGG AACGCGCTTC GGCTCGGACG CTGGCATAAT GGTCGTAAA

(67) INFORMATION FOR SEQ ID NO 66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 210

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Pseudallescheria boydii* region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

GACCAGACTT GTGCCCGTCG AATCAGCCGC CGCTCGTCGG CGGCGCACTT
CGGCGGGCTC AGGCCAGCAT CAGTTCGCTG CAGGGGGAGA AAGGCGATGG
GAATGTGGCT CTTCGGAGTG TTATAGCCCG CCGCGCAATA CCCCTCGGCG
GACTGAGGAC CGCGCATCTG CAAGGATGCT GGCGTAATGG TCGTCAGCGA
CCCGTCTTGA

(68) INFORMATION FOR SEQ ID NO 67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 244

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Rhizopus* species (NO: 1) specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

AGCCAGACTG GCTTGTCTGT AATCAATCTA GGTTTCGTGC CTGGATGCAC
TTGCAGACTA TTTGCCTGCC AACGACAATT TTTTTTGAGT GTAAAAACTA
TTGGAAATGT GGCCAATATT TATTTATTGG TGTTATAGTC CTTTAGAAAA
TACCTTGAAT TGGATTGAGG AACGCAGCGA ATGCTTCTCT TTnGAGGCAA
AGTCTTTTAT TGGGATTTAC GGATCAGACT GTGGCATTGT CACA

(69) INFORMATION FOR SEQ ID NO 68:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 215

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Rhizopus* species (NO: 2) specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

```
AGCCAGACTG GCTTGTCTGT AATCAATCTA GGCTTCGGCC TGGATGCACT
TGCAGGCTAT GCCTGCCAAC GACAATTTGA CTTGAGGGAA AAAACTAGGG
GAAATGTGGC CCACTTGTGG GTGTTATAGT CCCTTAGAAA ATACCTTGGG
TTGGATTGAG GAACGCAGCG AATGCTTATT GGCGAGTTTT CCAGGAAGGT
TTTCTGAGGT ACTAC
```

(70) INFORMATION FOR SEQ ID NO 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 215

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Rhizopus* species (NO: 3) specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

AGCCAGACTG GCTTGTCTGT AATCAGTCTA AGCTTCGGCT TGGATGCACT
TGCAGGCTAT GCCTGCCAAC GACAATTTGG CTTGAGGGAA AAAACTAAGG
GAAATGTGGC CCATCCGTGG GTGTTATAGT CCCTTAGAAA ATACCTTGGG
CTGGATTGAG GTACGCAGCG AATGCTATTT GGCGAGTTGG CTGGGAATAT
TTTCTGAGGT GCTTT

(71) INFORMATION FOR SEQ ID NO 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 210

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Sporothrix* species specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

GACCAGACTT GCGCCYCGCG GACCACCCGG CGTTCTCGCC GGTGCACTCT
GCGKKGCGCA GGCCAGCATC GGTTCTCCCA GGGGGACAAA GGCCGCGGGA
ACGTAGCTCC TTCGGGAGTG TTATAGCCCG CGGCGGCATG CCCCTGGGGG
GACCGAGGAC CGCGCTTCGG CAAGGATGCT GGCGTAATGG TCACCAGCGA
ACCGTCTTGA

(72) INFORMATION FOR SEQ ID NO 71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 208

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Scopulariopsis brevicaulis* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

GACCAGACTT GCGCCCGTCG GATCAACCGT CGCTTGCGGC GGCGCACTCC
GGCGGGCTCA GGCCAGCATC AGTTCGTCCG GGGGGAGAAA GGCGGCGGGA
ATGTGGCTCT TCGGAGTGTT ATAGCCCGCC GTGTAATACC CTCGGGTGGA
CTGAGGACCG CGCGTATGCA AGGATGCTGG CGTAATGGTC GTCAGCGACC
CGTCTTGA

(73) INFORMATION FOR SEQ ID NO 72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 210

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Scopulariopsis brumptii* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

GACCAGACTC GCGCCCGTCG GATCAGCCGT CGCTCGTCGG CGGCGCACTC
CGGCGGGCTC GGGCCAGCAT CAGTTCGCCT CGGGGGGAGA AAGGCGGCGG
GAATGTGGCT CTACGGAGTG TTATAGCCCG CCGCGTAATA CCCCCGGGCG
GACTGAGGAC CGCGCGTATG CAAGGATGCT GGCGTAATGG TCGTCAGCGA
CCCGTCTTGA

(74) INFORMATION FOR SEQ ID NO 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 214

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Saccharomyces cerevisiae* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

GATCAGACAT GGTGTTTTGT GCCCTCTGCT CCTTGTGGGT AGGGGAATCT
CGCATTTCAC TGGGCCAGCA TCAGTTTTGG TGGCAGGATA AATCCATAGG
AATGTAGCTT GCCTCGGTAA GTATTATAGC CTGTGGGAAT ACTGCCAGCT
GGGACTGAGG ACTGCGACGT AAGTCAAGGA TGCTGGCATA ATGGTTATAT
GCCGCCCGTC TTGA

(75) INFORMATION FOR SEQ ID NO 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 236

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: *Trichosporon beigelii* specific region of 28S gene.

(iii) HYPOTHETICAL: No

(iv) ANTISENSE: No

(v) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

AGTCAGTCGT GTTCTTTGGA TTCAGCCAGT TCTGCTGGTC TACTTCCTTG
GAACGGGTCA ACATCAGTTT TGTCCGGTGG ATAAAGGTAG TAGGAATGTG
ACTTCTCCGG AAGTGTTATA GCCTATTATC ACATACACTG GGTGAGACTG
AGGACTGCAG CTCGCCTTTA TGGCCGGCCT TCGGGCACGT TCGAGCTTAG
GATGTTGACA TAATGGCTTT AAACGACCCG TCTTGA

We claim:

1. An oligonucleotide probe for the 28S subunit of fungi which is able to identify one species selected from the group consisting of *Acremonium* sp., *Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Aspergillus unguis, Aspergillus ustus, Beauveria* sp., *Bipolaris* sp., *Blastoschizomyces* sp., *Blastomyces dermatitidis, Candida albicans, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Chrysosporium* sp., *Cladosporium* sp., *Coccidioides immitis, Cryptococcus neoformans var gattii* serotype B, *Cryptococcus neoformans* serotype A, *Cryptococcus laurentii, Cryptococcus terreus, Curvularia* sp., *Fusarium* sp., *Filobasidium capsuligenum, Filobasidiella* (Cryptococcus) *neoformans var bacillispora* serotype C, *Filobasidiella* (Cryptococcus) *neoformans var neoformans* serotype D, *Filobasidium uniguttulatum, Geotrichum* sp., *Histoplasma capsulatum, Malbranchea* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium species, Pseudallescheria boydii, Rhizopus* sp., *Sporothrix schenkii, Scopulariopsis brevicaulis, Scopulariopsis brumpti, Saccharomyces cerevisiae,* and *Trichosporon beigelii.*

2. An oligonucleotide probe of claim 1 comprising all or part of any one of the sequences (SEQ ID NO: 3) through (SEQ ID NO: 74), or any functional equivalent thereof, which is able to identify the corresponding species of fungus.

3. An oligonucleotide probe of claim 1 comprising all or part of any one of the sequences (SEQ ID NO: 3) through (SEQ ID NO: 74), or any functional equivalent thereof, which is able to identify any one or more of said species of fungus.

4. A method of determining whether one or more fungal species selected from a group of fungi is present in a sample comprising the following steps:

a. extracting the nucleic acid material from the fungi contained in said sample,

b. adding two known primers, (SEQ ID NO 1) and (SEQ ID NO 2), or the functional equivalent thereof, bracketing the areas of interest on the 28S rDNA or rRNA present in said group of interest,

c. amplifying the sequence between said primers, and

d. using one or more third labeled probes to determine which of said fungi is present, wherein said third probes are selected from the group consisting of (SEQ ID NO 3) through (SEQ ID NO 74), any portion thereof and functional equivalents thereof.

5. A method of claim 4 in which said amplifying procedure is the polymerase chain reaction.

6. A method of claim 4 which, following said amplification, comprises the following step:

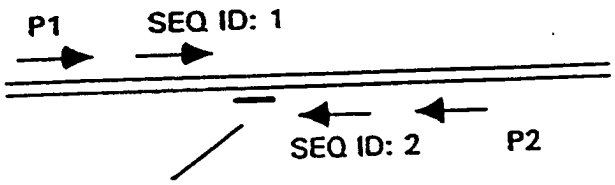
d. using two or more third probes to determine which of said fungi is present, one of said third probes being attached to a moiety which allows separation of said probe and one or more third probes connected to labeled moieties, wherein said third probes are selected from the group consisting of (SEQ ID NO 3) through (SEQ ID NO 74), any portion thereof and functional equivalents thereof.

7. A method of claim 4 which excludes said amplification step.

8. A method of claim 4 wherein said fungal species is selected from the group consisting of *Acremonium* sp., *Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Aspergillus unguis, Aspergillus ustus, Beauveria* sp., *Bipolaris* sp., *Blastoschizomyces* sp., *Blastomyces dermatitidis, Candida albicans, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Chrysosporium* sp., *Cladosporium* sp., *Coccidioides immitis, Cryptococcus neoformans var gattii* serotype B, *Cryptococcus neoformans* serotype A, *Cryptococcus laurentii, Cryptococcus terreus, Curvularia* sp.,

Fusarium sp., *Filobasidium capsuligenum, Filobasidiella* (Cryptococcus) *neoformans var bacillispora* serotype C, *Filobasidiella* (Cryptococcus) *neoformans var neoformans* serotype D, *Filobasidium uniguttulatum, Geotrichum* sp., *Histoplasma capsulatum, Malbranchea* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium species, Pseudallescheria boydii, Rhizopus* sp., *Sporothrix schenkii, Scopulariopsis brevicaulis, Scopulariopsis brumpti, Saccharomyces cerevisiae,* and *Trichosporon beigelii.*

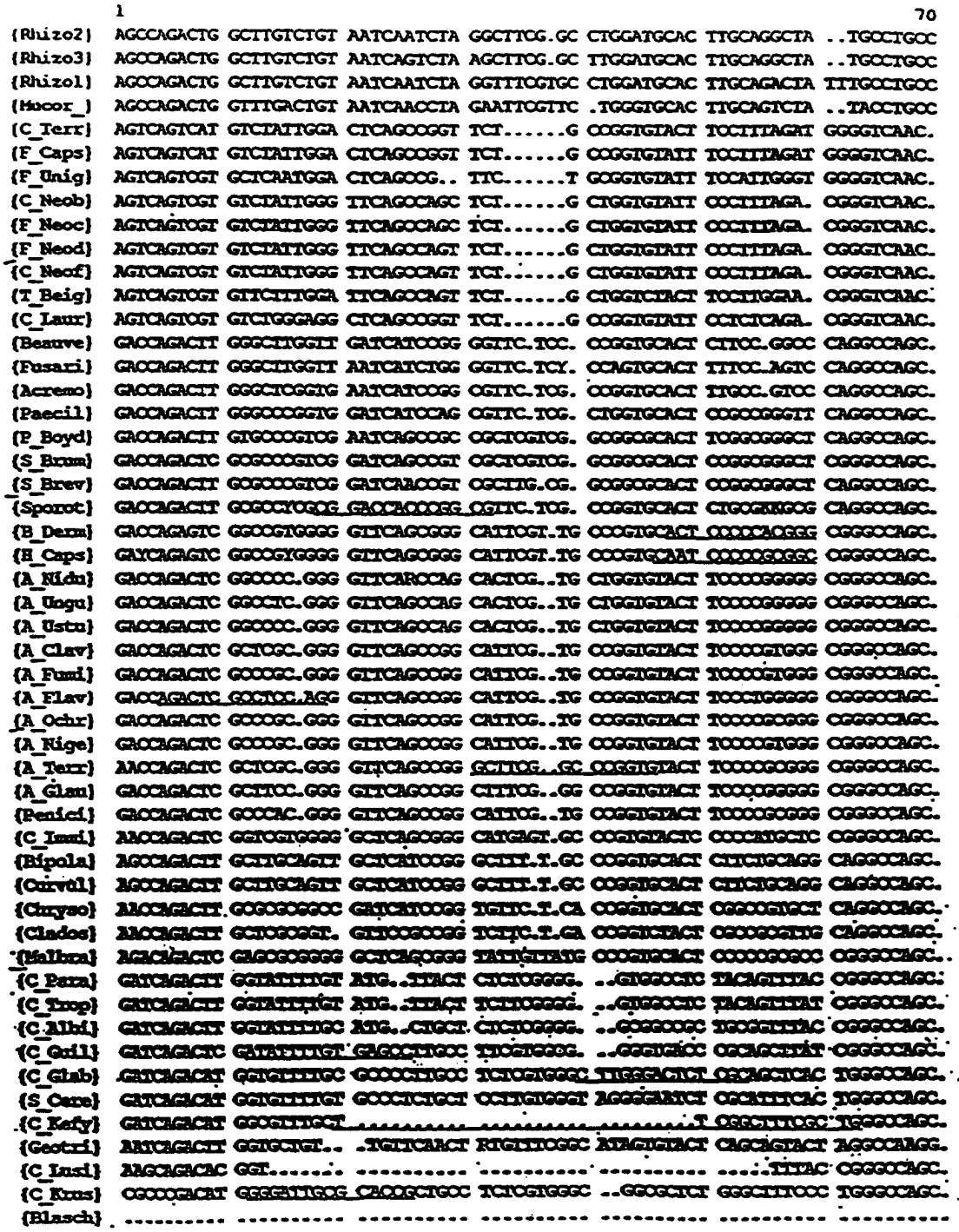
9. A method of claim 4 wherein more than one third probe is used, each said third probe connected to a different signal moiety or moiety which allows separation of said third probe.

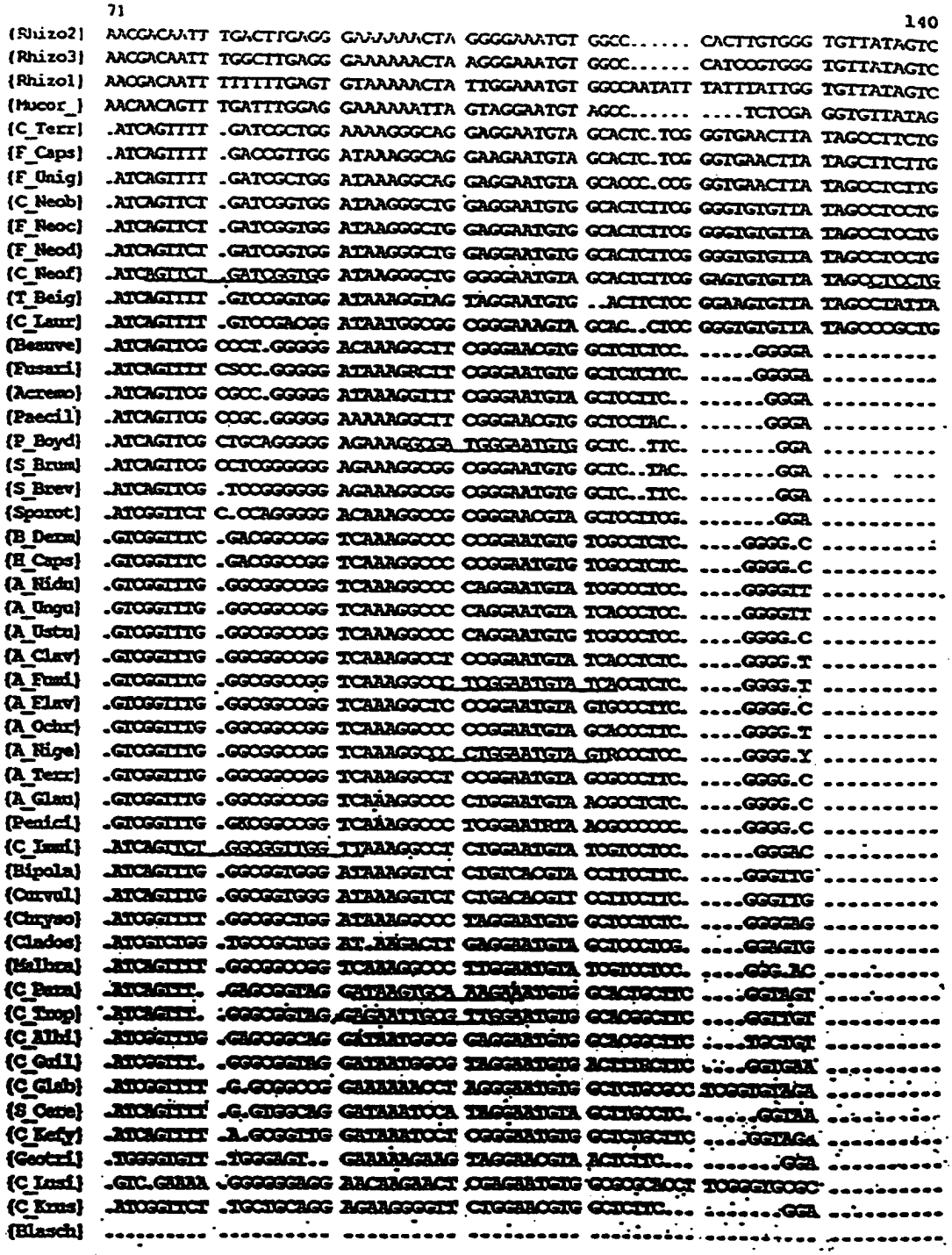


SEQ ID: 3 to SEQ ID: 23

FIGURE 1

Figure 2A





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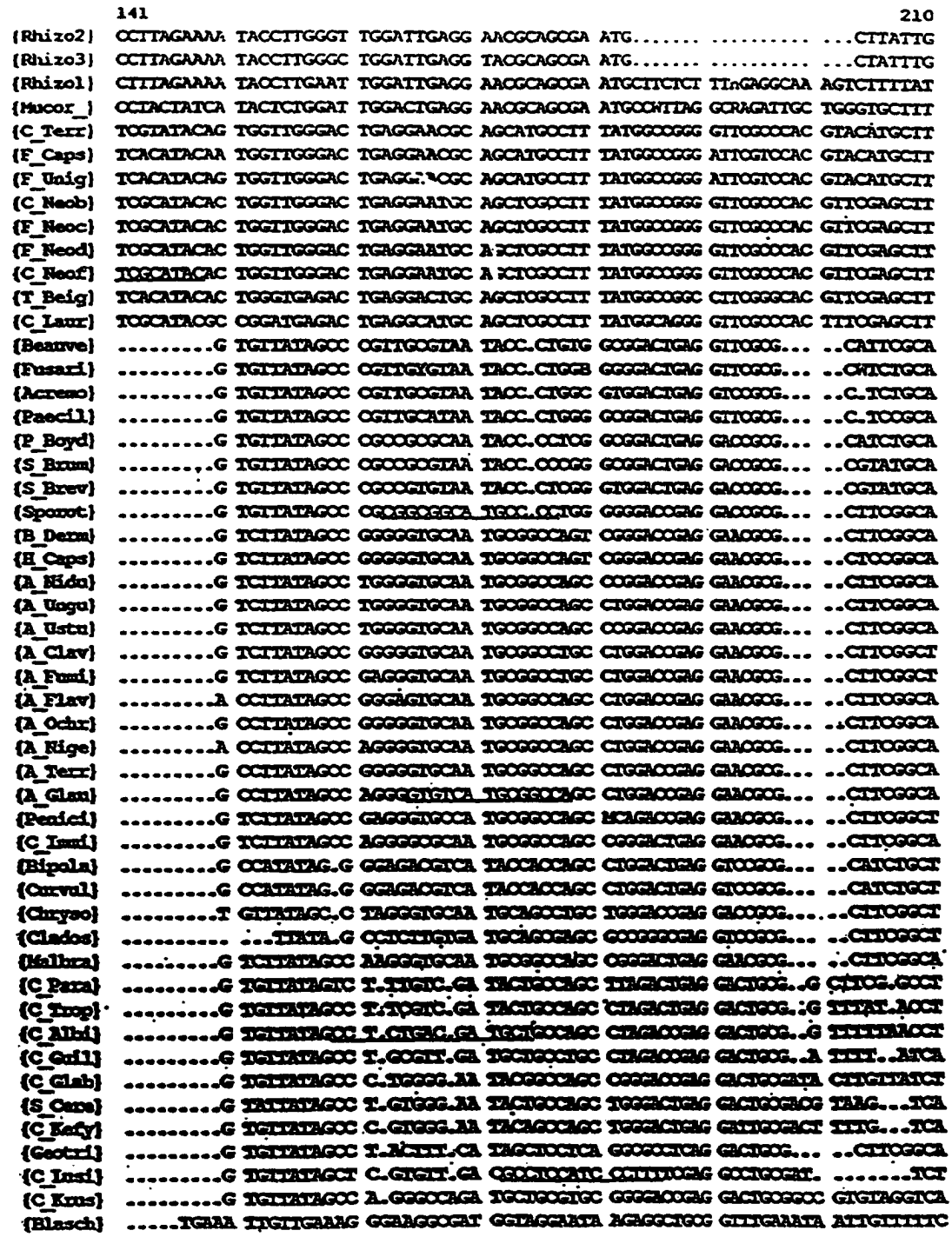
SUBSTITUTE SHEET (RULE 26)



```
           211                                                  250
{Rhizo2}   GCGAGTTTTC CAGGAAGGT. .....TTTCT GAGGTACTAC
{Rhizo3}   GCGAGTTGGC TGGGAATAT. .....TTTCT GAGGTGCTTT
{Rhizol}   TGGGATTTAC GGATCAGAC. .....TGTGG CATTGTCACA
{Mucor_}   CGCTAATAAA TGTTAGAATT TCTGCTTCGG GTGGTGCTAA
{C_Terr}   AGG..ATGTT GACATAATGG CTTTAAACGA CCGGTCTTGA
{F_Caps}   AGG..ATGTT GACATAATGG CTTTAAACGA CCGGTCTTGA
{F_Unig}   AGG..ATGTT GACATAATGG CTTTAAACGA CCGGTCTTGA
{C_Neob}   AGG..ATGTT GACAAAATGG CTTTAAACGA CCGGTCTTGA
{F_Neoc}   AGG..ATGTT GACAAAATGG CTTTAAACGA CCGGTCTTGA
{F_Neod}   AGG..ATGTT GACAAAATGG CTTTAAACGA CCGGTCTTGA
{C_Neof}   AGG..ATGTT GACAAAATGG CTTTAAACGA CCGGTCTTGA
{T_Beig}   AGG..ATGTT GACATAATGG CTTTAAACGA CCGGTCTTGA
{C_Laur}   AGG..ATGTT GACGTAATGG CTTTAAACGA CCGGTCTTGA
{Beauve}   AGG..ATGCT GGCGTAATGG TCATCAGTGA CCGGTCT...
{Fusari}   AGG..ATGCT GGCGTAATGG TCATCAACGA CCGGTCTTGA
{Acremo}   AGG..ATGCT GGCGTAATGG TCATCAGTGA CCGGTCTTGA
{Paecil}   AGG..ATGCT GGCGTAATGG TCATCAGCGA CCGGTCTTGA
{P_Boyd}   AGG..ATGCT GGCGTAATGG TCGTCAGCGA CCGGTCTTGA
{S_Brum}   AGG..ATGCT GGCGTAATGG TCGTCAGCGA CCGGTCTTGA
{S_Brev}   AGG..ATGCT GGCGTAATGG TCGTCAGCGA CCGGTCTTGA
{Sporot}   AGG..ATGCT GGCGTAATGG TCACCAGCGA ACGGTCTTGA
{B_Derm}   CGG..ACGCT GGCTTAATGG TCGTAAGCGA CCGGTCTTGA
{H_Caps}   CGG..ACGCT GGCTTAATGG TCGTCAGCGA CCGGTCTTGA
{A_Nidu}   CGG..ACGCT GGCGTAATGG TCGCAAACGA CCGGTCTTGA
{A_Ungu}   CGG..ACGCT GGCATAATGG TTGCAAACGA CCGGTCTTGA
{A_Ustu}   CGG..ACGCT GGCGTAATGG TCGCAAACGA CCGGTCTTGA
{A_Clav}   CGG..ACGCT GGCGTAATGG TCGTAAATGA CCGGTCTTGA
{A_Fumi}   CGG..ACGCT GGCGTAATGG TCGTAAATGA CCGGTCTTGA
{A_Flav}   CGG..ACGCT GGCATAATGG TCGTAAACGA CCGGTCTTGA
{A_Ochr}   CGG..ACGCT GGCATAATGG TCGTAAACGA CCGGTCTTGA
{A_Nige}   CGG..ACGCT GGCATAATGG TCGTAAACGA CCGGTCTTGA
{A_Terr}   CGG..ACGCT GGCATAATGG TTGTAAACGA CCGGTCTTGA
{A_Glau}   CGG..ACGCT GGCATAATGG TCGTAAACGA CCGGTCTTGA
{Penici}   CGG..ACGCT GGCATAATGG TCGTAAA... ..........
{C_Immi}   CGG..ATGCT GGCATAATGG TTGTAAGCGG CCGGTCTTGA
{Bipola}   AGG..ATGCT GGCGTAATGG CTGTAAGCGG CCGGTCTTGA
{Curvul}   AGG..ATGCT GGCGTAATGG CTGTAAGCGG CCGGTCTTGA
{Chryso}   AGG..ATGCT GGCGTAATGG TTGTAAGCGG CCGGTCTTGA
{Clados}   AGG..ATGCT GGCGTAATGG TCGTAATCGG CCGGTCTTGA
{Malbra}   CGG..ATGCT GGCGTAATGG CTGTAAGCGG CCGGTCTTGA
{C_Para}   AGG..ATGTT GGCATAATGA TCTTAAGTCG CCGGTCTTGA
{C_Trop}   AGG..ATGTT GGCATAATGA TCTTAAGTCG CCGGTCTTGA
{C_Albi}   AGG..ATGTT GGCATAATGA TCTTAAGTCG CCGGTCTTGA
{C_Guil}   AGG..ATGCT GGCATAATGA TCCCAAACGG CCGGTCTTGA
{C_Glab}   AGG..ATGCT GGCATAATGG TTATATGCCG CCGGTCTTGA
{S_Cere}   AGG..ATGCT GGCATAATGG TTATATGCCG CCGGTCTTGA
{C_Kefy}   AGG..ATGCT GGCGTAATGG TTAAATGCCG CCGGTCTTGA
{Geotri}   AGG..ACGTT GGCATAATGA TTCTATACCG CCGGTCTTGA
{C_Inni}   AGG..ACGCT GGCGTAATGG TTGCAAGCCG CCGGTCTTGA
{C_Krus}   CGG..ATGCT GGCAGAACGG CGCAACACCG CCGGTCTTGA
{Blasch}   GGGCCACGGT CTCTTGAGCC TGCTTTCGCA CCGGTCTTGA
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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 96/00026

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF MEDICAL AND VETERINARY MYCOLOGY, 32 (5). 1994. 331-341., XP002002929 LECLERC M C ET AL: "Phylogeny of dermatophytes and dimorphic fungi based on large subunit ribosomal RNA sequence comparisons" cited in the application	1
A	see the whole document ---	2-9
X	CURRENT GENETICS, 27 (1). 1994. 38-45., XP002002930 NEUVEGLISE C ET AL: "Identification of group-I introns in the 28s rDNA of the entomopathogenic fungus Beauveria brongniartii"	1
A	see the whole document ---	2-9
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
21 May 1996	04.06.1996

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Gurdjian, D

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INTERNATIONAL SEARCH REPORT

Inter vnal Application No
PCT/IB 96/00026

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PHYTOPATHOLOGY, 84 (3). 1994. 256-259., XP002002931 MOUKHAMEDOV R ET AL: "Use of polymerase chain reaction-amplified ribosomal intergenic sequences for the diagnosis of Verticillium tricorpus"	1
A	see the whole document	2-9
X	CURR GENET, 12 (3). 1987. 209-214., XP002002932 CARR L G ET AL: "ORGANIZATION OF THE 5.8S 16-18S AND 23-28S RIBOSOMAL RNA GENES OF CEPHALOSPORIUM-ACREMONIUM"	1
A	see the whole document	2-9
X	JOURNAL OF BACTERIOLOGY, vol. 172, no. 8, 1990, pages 4238-4246, XP002002933 VILGALYS R. ET AL.: "Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species"	1
A	see the whole document	2-9
A	US,A,5 352 579 (MILLIMAN CURT L) 4 October 1994 see the whole document	2-9
A	EP,A,0 422 872 (GENE TRAK SYSTEMS) 17 April 1991 see the whole document	2-9
P,X	JOURNAL OF CLINICAL MICROBIOLOGY, 33 (11). 1995. 2913-2919., XP002002934 SANDHU G S ET AL: "Molecular probes for diagnosis of fungal infections" see the whole document	1-3

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 96/00026

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5352579	04-10-94	NONE		
EP-A-0422872	17-04-91	AU-B-	6390490	18-04-91
		CA-A-	2025181	13-04-91
		JP-A-	3168085	19-07-91
		US-A-	5324632	28-06-94